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THE METHOD OF CELL DIVISION IN THE DEVELOPMENT OF THE FEMALE SEX ORGANS OF MONIEZIA.[1]

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INTRODUCTION.

Cestode histology has been the subject of several investigations in recent years, some of the results of which are of far-reaching importance. Throughout the development of *Moniezia* Child has reported the regular occurrence of amitosis; he has described it as present not only in the somatic tissues but also in the germ cells. It was this latter phase of his results which occasioned the present work.

[1] Zoölogical Laboratory, Princeton University, January 26, 1911.

There are inherent in the very nature of the problem involved in the recognition of cases of amitotic cell division as such certain difficulties which render the study of the question upon fixed material more or less unsatisfactory. Mitoses are, of course, well adapted for such a study, although they may be obscured in various ways. The presence of definite chromatin bodies and the absence of a nuclear membrane at most stages makes their recognition in properly treated material comparatively easy. But positive evidence of amitosis in fixed material is difficult to obtain. One cannot always be sure that a nuclear constriction is not a product of mechanical compression, *e. g.*, by yolk granules, or an artifact due to reagents rather than a stage in division. A strand of linin stretched across a nucleus with chromatin granules upon it often gives the appearance of a membrane dividing the nucleus amitotically (endogenous division?). Nuclear lobations are frequently seen in cells which are known to multiply by mitosis. Finally, a reduplication of nuclei for any of several reasons—disintegration, accessory sperm nuclei in egg cells, the male and female pronuclei themselves, lagging of cytoplasmic division behind nuclear mitosis, incompletely fused chromosomal vesicles, etc., easily presents a simulacrum of amitosis which is quite misleading. These and other factors[1] all render the amitosis problem difficult of solution.

Clearly, however, a definition of what one means by amitosis and of what he will accept as evidence of that process is imperative. Dahlgren and Kepner (p. 38) define amitosis as division "by a series of autoconstrictions of first the nucleolus, then the nucleus, and lastly the cytoplasmic body." The nucleus itself divides in three ways: the constriction of its body, the formation of a "nuclear plate" in the plane of division and subsequent separation, and the endogenous method of Child. Wilson (p. 114) describes the process as follows: "First the nucleus remains in the resting stage (reticulum), and there is no formation of a spireme or of chromosomes. Second, division occurs without the formation of an amphiaster. . . . The nuclear substance undergoes a division of its total mass but not of the individual elements or chromatin granules." It will be seen that these

[1] Mitosis may be periodic and of very short duration.

definitions are not sufficiently diagnostic to be of use in the work here undertaken.

For amitosis there is but one absolutely certain criterion, the observation of living material and subsequent study of material fixed under observation, and this is, of course, impossible in most cases. In the absence of such a criterion only complete series of all stages in the constriction and subsequent division of the nucleus and attendant cytoplasm would seem sufficient warrant for the assertion that amitosis is the prevailing form of division.[1] Even complete series are no assurance that part of the cells do not divide mitotically or that mitosis does not occur periodically. But given a complete series—assumed or proven—and taking no account of periodicity, etc., the interpetation of doubtful cases on the basis of the complete series follows logically. As will appear later such a complete series is extremely difficult to establish in the cestodes; its assumption, therefore, in the face of the above mentioned difficulties is ultra radical. The burden of proof is upon the shoulders of him who makes the assumption.

It is true, however, that a spirit of open-mindedness demands an impartial consideration of uncertain cases, incomplete stages and doubtful processes in the hope that an unprejudiced decision may be reached. The refusal to consider such cases is as unscientific as is the drawing of sweeping generalizations from them. In such a spirit of investigation I propose to set forth the facts as I see them in the case of the anlage of the female sex organs and its derivatives in *Moniezia*. I then wish to sum up the evidence for and against the occurrence of amitosis in this form and to consider various *a priori* suggestions which have been made by investigators to cover this case. Finally, I wish to make a comparison of these results with those of *Tænia* upon which I previously worked. In the development of the anlage of the female sex organs the history of the sex cells themselves, of the vitellarium and of the ducts and canals will be considered.

Interesting in this connection are the recent comments upon Child's position made by Boveri.[2]

[1] To forestall criticism of this point, let me say that this is in no sense a preconceived hypothesis. I had worked on the amitosis question nearly two years before the significance of this contention was fully borne in on me. It is given thus early in the discussion merely to make the account more intelligible.

[2] "Zellenstudien," 6, p. 235.

"If, his (Child's) observations are to prove his contentions then it must be shown: (1) that the binucleate condition which he finds really depends on a division; (2) that about each of these nuclei a part of the protoplasm is marked off, and (3) that the cells so arising again divide by mitosis and possess the normal number of chromosomes."

Review of the Literature.

The literature of late histological researches upon the trematodes and cestodes deals with the general development of the flat-worm tissues, especially with the cuticle and the parenchyma. The more recent papers also touch upon the question of the occurrence of amitosis in the life history of the various cells.

Cestode histology according to this late work presents some very unique features. All investigators who have given the subject their attention seem to concur in the view that tissue growth here differs in character from the usual method of cell multiplication. All agree that karyokinetic figures are not to be found as numerously as in other tissues; some assert their entire absence; while one goes so far as to claim that nuclei arise *de novo*. On the other hand, some papers make no mention of amitosis, possibly due to the fact that the authors have not interested themselves with the problem. A comparison of the cases in which amitosis occurs is difficult to make because many reports, of thirty or more years ago, previous to the introduction of refined methods of technique, have not been substantiated by later work; but, nevertheless, it is safe to say that in no other group of animals have such unique growth phenomena been found by reputable workers. Granting for the moment that these phenomena have been correctly reported, one is compelled to ask what the relation is between them and the parasitic mode of existence which is universal among cestodes. While as yet there is no basis for speculation even on this relation, the modifications of gross structure due to parasitism are so profound in the cestodes that one may well be deterred from generalizing from observations of peculiar histological conditions in them.

Bugge ('02) was the first to note the absence of mitosis in cestode tissues in his work upon the flame cells.

Child in 1904 called attention to the lack of mitoses in the reproductive organs of the cestode *Moniezia* and asserted that amitosis takes its place. He further elaborated this conception and furnished more data in 1906 and 1907. As the object of this research is either to corroborate or to disprove his conclusions, and as it deals with them at length later, no further attention will be given to his earlier papers in this place. In a later paper ('10) he brings forward no new evidence but merely reiterates his former position. This work consists largely of an attack on my observations on *Tænia* ('09). He criticises my paper as presenting evidence of a negative character only; as being upon an entirely different genus and perhaps on different stages from those in which he observed amitosis; as prematurely generalizing from incomplete data; as using preconceived hypotheses; and finally, as being the work of a novice who "is likely to be guided to a greater or less extent by the views of his instructor." I may venture a reply to the main points of this list. I regret to have my paper taken solely as a criticism of Child's work; it recounted the facts as I believe them to exist in *Tænia;* its field, it is true, is limited to the oögenesis and cell multiplication of certain somatic tissues in this worm, but no attempt is made to apply its conclusions to any of Child's work not upon a similar field (a fact which he does not seem to have appreciated). I found no evidence of amitosis in that field and as Child had covered a similar one it was not only natural but necessary that I should compare my results with his. As to the negative character of my evidence, I would ask whether the failure to find evidence of a certain process, using proper methods and exercising due diligence, is not positive evidence of the lack of that process. Of what more would positive evidence consist? As to my not having studied the proper stages little need be said; no method of procedure other than beginning at the scolex and working backward ever occurred to me. As to the fact that I studied a different genus, he says, "Such records (referring to the results published in his previous papers) cannot be controverted . . . by observation upon another genus and species. . . ." His views upon this point, it seems, have undergone a change. In his "General Conclusions" to "Amitosis in Moniezia" ('04) he said, "It is

scarcely possible that *Moniezia* differs from the majority of other forms in the amitotic multiplication of nuclei." There are, however, a considerable number of differences in the results obtained in the two allied forms, differences which I hope to make clear in the course of this discussion.

Spengel in 1905 before the Deutsche Zoologische Gesellschaft[1] stated that neither mitotic nor amitotic cell division had been observed in cestode development.

C. v. Janicki ('07) in an important paper upon the embryonic development of *Tænia serrata* states that he finds no pronounced division figures up to the growth period of the oögonia, but during the growth period he describes spiremes; he does not suggest amitosis as the explanation of his failure to find mitoses but merely says that the divisions must have passed very quickly. In the cleavage stages achromatic parts of the division figures are often hard to demonstrate.

In so far as it touches the same points my own work ('09) on this form agrees with that of v. Janicki. I recorded my failure to find amitosis and noted that mitoses are more infrequent than one would expect.

In 1908 appeared the paper by Young upon the histogenesis of *Cysticercus pisiformis*, the cysticerus stage of *Tænia serrata*. This paper is revolutionary indeed, and agrees in practically no cytological points with the work of v. Janicki and myself. Not only does he accept Child's conclusions and their bearing upon hereditary problems but he refuses to subscribe to Virchow's dictum "omnis cellula e cellula." He believes that nuclei arise *de novo* in accordance with a tentative hypothesis, of which his statement is as follows: "I believe that the nucleus in these forms is not a morphological but a physiological entity; that the nuclear granules are fundamentally the same as the remaining protoplasm of the cell, but are differentiated therefrom under physiological conditions which we do not at present understand; that these granules are perhaps reserve material stored up in the nucleus for future use, the entire cell body being occasionally converted into a nucleus; and that the nucleus varies in structure from time to time in response to the varying physiological de-

[1] See Child, '07*a*, p. 277.

mands made upon it. . . . Further, if my interpretation of my observations be correct, then distinction between germ and somatic plasm is obviously impossible; a special vehicle for the transference of heredity qualities is entirely wanting; such qualities must be transmitted by the undifferentiated protoplasm; cell lineage is manifestly lacking; a mosaic theory is plainly untenable; and the fate of any given embryonic element—whether it shall form parenchyme, muscle, nerve, etc.—must be determined by physiological causes alone." In an appendix referring to Child's later papers Young says that he has verified Child's conclusions upon the reproductive organs of *Tænia serrata*. This, of course, is exactly opposed to the work of v. Janicki and myself upon *T. serrata*. I am not aware that he has published any further account of these more recent observations.[1]

Balss ('08), in his extensive researches on the development of the sex ducts of *Anoplocephala* and *Solenophorus* did not, I believe, direct his attention to the method of nuclear division, nor do his figures throw any light upon the question. His paper is extremely valuable in making a study of the early stages of sex organ formation.

The last of the important papers on cestode histology is that of Spätlich ('09) on *Tetrabothrius*. This author states that he finds cases of amitosis and gives several figures of the cells in question. He does not doubt that the cells are in the process o amitotic division and certainly that is the easiest explanation of the figures; but to one skeptically minded the proof will not appear as conclusive. The cell in his Fig. 54, for example, might easily be interpreted as a case of compression; while there is no evidence that either of Figs. 54, 55 and 56 (drawn out nuclei, becoming dumb-bell shaped) would actually have completed the division. Nothing is said of a complete series of stages. Furthermore, these are cases in the development of the "Dotterzelle" and may perhaps have been at the end of their life cycle.

In glancing over the list of tape-worms in which amitosis or other irregularities of cell multiplication have been described, one

[1]In *Science* of February 17, Young gives a very brief résumé of this work. Although he finds no mitosis, too few amitoses occur to account for the necessary cell multiplication; he thinks that the "increase is partly due to development of nuclei either de novo or from chromidial extrusions of preexistent nuclei."

is struck with the fact that according to the classification usually given only members of one family, the Tænidæ, are included in it. This, of course, may be the merest coincidence owing, perhaps, to the fact that members of the Tænidæ are very readily secured. The warning uttered by Young with regard to applying conclusions drawn from cestode observations to other groups, is, however, more strongly emphasized. Not only are the results which he had in mind deduced from a single class of animals which are degenerate in a high degree, but they are drawn from a single family of that degenerate class.

Material and Methods.

At the suggestion of Professor E. G. Conklin, the genus *Tænia*, upon which I had begun investigation with a view to obtaining further evidence as to the occurrence of amitosis in cestode tissues, was dropped for the present as a subject for research in favor of *Moniezia*. The results which have been obtained upon this latter genus by Child are of such unusual nature compared with those of other forms that a reëxamination would seem desirable. In the light of my investigations upon *Moniezia* and of Child's recent criticism I have again gone over my old material of *Tænia* as well as some newly prepared.

In making these studies I am indebted to Professor E. G. Conklin and to Professor Ulric Dahlgren for much kindly assistance. My thanks are due to the management of the Wistar Institute of Anatomy for assistance in obtaining material. Not only were the facilities of the laboratory extended me for making collections but my first lot of material was obtained and fixed by Dr. Helen Dean King. To Dr. King I also owe thanks for a number of technical suggestions. Professor C. M. Child, of the University of Chicago, has also kindly placed facilities at my disposal for obtaining specimens.

Specimens of *Moniezia expansa* and of *M. planissima* have been secured and fixed in a variety of fixing fluids: Flemming, Hermann, Graf's chrom-oxalic, saturated aqueous solution of corrosive sublimate, formol-sublimate,[1] Kleinberg's picro-sul-

[1] Physiological salt solution saturated with corrosive sublimate to which a few drops of neutral formol are added.

phuric, Zenker's fluid, Zenker plus an excess of acetic acid, and a modification of Zenker's fluid by Dr. King.[1]

None of my sections have been cut over 4 micra thick; many of them are thinner. Pieces of the worms about 3–6 mm. in length were embedded serially beginning at the scolex or the neck region and extending back some 35–40 cm. usually, including the beginning of the proglottids with fully formed embryos. Pieces were then selected at intervals and cut; later, if examination warranted, the intervening pieces were cut. In this way practically one entire worm has been cut and studied, while from a considerable number of other worms incomplete series have been made as described. I have used both longitudinal and cross sections.

A number of staining methods have been tried on *Moniezia*, some of which gave good results, while others succeeded only indifferently. I have been unable to get good preparations with Kernschwarz, although this stain served me well on *Tænia*. The other stains used include Delafield's hæmatoxylin, hæmalum, thionin, Bismarck brown, iron hæmatoxylin alone and counterstained with Lichtgrün or Bordeaux R, a modification of Conklin's picro-hæmatoxylin method for staining eggs *in toto*,[2] Ehrlich-Biondi, and the Flemming triple stain, safranin gentian-violet and orange G.

Of these stains, iron hæmatoxylin is the best general stain and most reliable, but for certain phases of the work it is not sufficiently differential. I have found picro-hæmatoxylin and the safranin gentian-violet mixture especially useful in connection with it. (In most cases I find that the orange G of the triple stain detracts from rather than adds to its value.)

[1] Dr. King uses two solutions, as follows: (1) Four per cent. potassium bichromate; (2) corrosive sublimate, 4 gr., glacial acetic acid, 20 c.c., water, 76 c.c. The two solutions are mixed equally and allowed to act for one to two hours as the mixture penetrates rapidly. For convenience, I shall hereafter call this mixture "King's fluid."

[2] This is an application of the *in toto* method to sections containing eggs. It consists of mordanting the sections for a short time—too long is injurious—in aqueous picric acid solution. After staining the sections may be decolorized in this same solution and then blued in tap water. It gives the desired differentiation between yolk and chromatin, although not with the characteristic colors of Conklin's stain.

Fixatives.—To show exactly the various effects of the fixatives used and to show that the results obtained are not due to the exclusive use of one reagent, I wish to give here a detailed statement of those effects.

In his work on *Moniezia* Child used some eleven fixatives of which the most successful were Hermann's fluid, saturated aqueous solution of corrosive sublimate, and Graf's chrom-oxalic mixture. I, therefore, used these three fluids and in addition certain others which my own experience suggested. These were, as previously stated, Flemming's and Zenker's fluids, Zenker plus an excess of acetic acid, and formol-sublimate. Dr. King fixed some of my first lot of material in her modification of Zenker's fluid. I have no hesitation in saying that this fluid gives the best results for *Moniezia* of any which I have tried. Subsequently, I myself have used this solution with equally satisfactory results.

The results of my experiments with the various fluids follow. Chrom-oxalic is not, I find, a good general fixative for *Moniezia*. The general cytoplasmic structures, parenchyma, etc., are not well preserved and the achromatic substances of the nuclei are dissolved out. The chalk bodies[1] also are not retained. Testis cells are more satisfactorily fixed, as are also the cleavage cells and young embryos. But in the case of spermatozoa and the young female germ cells the mixture is not successful. Nuclei are everywhere swollen, but in these special cases they are also distorted. I should not care to trust any results based on the observation of early oögonia in my chrom-oxalic material. Many of Child's results are founded on chrom-oxalic material; doubtless he had greater success with this mixture than I did.

Hermann's is a good fixative for the developing germ cells, both male and female; for parenchyma the results are slightly less satisfactory. Nuclear contents are well preserved in this solution—much better than in the preceding one. It also gives a good preparation of the cuticle. With Flemming's, Hermann's shares the drawback of fixing the calcareous bodies so that they

[1] As part of their "generic diagnosis" Stiles and Hassal give this statement, "Calcareous bodies absent from the parenchyma." They, however, studied only sublimate material.

stain black with certain dyes. This is a serious drawback to the use of osmic acid fixatives, for the chalk bodies are so numerous as to obscure much of the development of the reproductive organs.

The remarks concerning Hermann's apply with equal force to Flemming's although the latter gives a somewhat more nearly perfect fixation considering the tissue as a whole. For the cuticle Flemming's gives better results than any other reagent I have tried.

My experiments with corrosive sublimate in aqueous solution have proven a complete failure. Circumstances connected with the experiments were such, however, that I am not disposed to regard my results as typical; had I not already had a great number of successful preparations I should have given it further trial.

With formol-sublimate I have obtained good preparations. It gives quite good nuclear and cytoplasmic fixation as well as a very fair preservation of the parenchyma. Nuclei and cytoplasm stain dark after it; chromatin is well fixed but achromatic structures are not easily differentiated owing to the homogeneous condition of the nuclei; cytoplasm, too, is of a dense homogeneous nature not unlike that of certain nerve cells. Calcareous bodies are not present in sublimate material. Formol-sublimate is, however, a good general fixative for *Moniezia*.

Picro-sulphuric, while giving somewhat better results than aqueous sublimate (the young embryos are fairly well preserved) is also a failure. It gives a better fixation of the parenchyma than of other tissues.

There yet remain the Zenker solutions. These give the best fixations, and of them the modification by Dr. King is the most satisfactory of all the fluids I have used on *Moniezia*. I find, too, that I am able to get better staining reactions on the material fixed in this solution. My study has been made chiefly on this material but I have in every case compared the results with those of the other fixatives. The fixations by chrom-oxalic, Hermann's, formol-sublimate, and King's solution all depend on different actions; therefore, it would seem that the phenomena common to these four reagents cannot be viewed as artifacts due to fixation.

Observations.

Morphological discussions are without the scope of this research, but a knowledge of the derivation is helpful to an understanding of the development of the structures dealt with. Excluding the cuticle—the writer has not given much attention to the "ectoderm question," but his view, in so far as his observations warrant one, is that the cuticle also is of parenchymal origin—all of the adult structures of the tape-worm are derived from the parenchyma. Parenchyma is physiologically similar to embryonic mesenchyme in that from it are derived muscle fibers, flame cells, etc. The testes arise from the parenchyma first in the region of the longitudinal excretory tubes and later appear medianward, occupying the side of the worm commonly designated as dorsal. The animal is incompletely protandrous for the ovaries arise later and many of the testes have completed their development and degenerated before the female germ cells have progressed very far. The last of them do not complete their cycle until most of the ova have already been fertilized but all ultimately degenerate and their place is taken by the branches of the uterus.

The first anlage of the female reproductive apparatus arises also in the neighborhood of the excretory tubes and grows both laterally and medianward. Its lateral growth gives rise to the seminal receptacle and the vagina. Its median growth is the beginning of the oviduct, which later branches to form the uterus, of the vitellarium and shell glands, and of the ovary. Thus the entire female apparatus arises from an originally single, indifferent anlage. This shortly differentiates into the separate anlagen each of which from now on pursues its own individual course. That of the ovary is the innermost part of the original anlage. It develops its investing membrane and begins a growth, due to the growth of the germ cells composing it, which ends only when the ova have all attained their complete size and developed the needed amount of yolk. These in a rapid passage through the oviduct are fertilized and enclosed in a thin shell. Maturation is completed in the uterus where cleavage occurs and early embryonic life is passed. When all of the ova have been fertilized the reproductive mechanism except the uterus degenerates. The

entire process of development from primitive germ cells to fully formed embryos is one of comparative simplicity and is quite easily followed with the exception of the cleavage stages. These are difficult to understand in section, and whole mounts of them are not readily prepared.[1]

The vitellarium arises from the middle part of the primary indifferent an age, and in its early stages cannot be distinguished from the ovary other than by its position. But its growth is of a different character and soon permits of a distinction between the cells of the two regions. It acquires the character of a gland and thereafter its activities are those connected with secretion.

The development of the various female organs will now be considered in detail.

The Primary Anlage.—The indifferent or primary anlage begins merely as a thickening of cells median and slightly ventral to the longitudinal nephridial canals. This thickening is not marked off in any way from the surrounding parenchyma; in fact there is a gradation from one into the other. There is a slight difference in the character of the cells of the an age and of the adjacent parenchyma but it is only slight. The cytoplasm is perhaps a little more dense in the anlage, and the nuclei show evidence of greater activity. The cells of the parenchyma are clear and usually have but one nucleus. The cells of the anlage stain darker, and their nuclei frequently possess two or more nucleolus-like bodies, of which probably only one is a true nucleolus, and show a more or less distinct reticulum, except when fixed with chrom-acetic. The ratio of nucleus to cytoplasm is at first somewhat greater but soon becomes less in the case of the anlage than of the parenchyma. Nuclei in the former are often somewhat smaller than in the latter.

To avoid confusion with regard to my use of the term "cell," I will here state my view of the cellular nature of the parenchyma. I do not consider the parenchyma to be a syncytium as that term is generally interpreted. Nuclei are always surrounded by a definite amount of cytoplasm, but that cytoplasm is lacking in a definite limiting membrane or cell wall.[2] The remainder of

[1] Cf. for this general development photomicrographs I. to IV.

[2] My former statement that "the parenchyma cells of *Tænia* have definite cell boundaries" was not intended to mean that they have cell walls; I have not observed that the two genera differ in this respect.

the parenchyma tissue is made up of loose intercellular material (of cellular origin, of course). Thus a cell consists of a nucleus and its attendant cytoplasm supported by a large mass of intercellular material. Usually the cells are somewhat distant from each other. Should two or more of them come to lie closely side by side, it is conceivable that their cytoplasmic masses might easily fuse and a true syncytium be formed. It will be seen that this is a possible explanation of binucleate parenchyma cells where such occur (Fig. 2). The cellular character of the primary anlage is similar to that of the parenchyma, although, of course, the anlage has very little intercellular material and many cells.

Child describes the testes as arising from visibly differentiated muscle cells; he did not, however, observe that muscle cells take part in the formation of the ovary, "probably because these cells do not occur in the region where the ovaries develop" (Child, '06). Muscle cells certainly do occur in the region of the ovaries[1] but I have never seen any evidence that they take any part whatever in the development of the female germ cells.

Since the primary anlage arises from a parenchyma which has no visible differentiation there would seem to be no visible basis for a continuity of germ plasm here. The expression "primordial germ cell" has little meaning therefore when applied to a cestode for there is no apparent difference, except of position,[2] in the cells which will go to form the ova and those which will form the vitellaria or the walls of the ducts.

The primary anlage grows both outward toward the edge of the proglottid and inward toward the ventral surface. Its outward growth is in the form of a cord of cells being extended to the exterior. Before the solid cord reaches the exterior its middle portion becomes hollowed out to form a lumen. This lumen subsequently develops a cuticle which to all appearances is just like that on the surface of the body. This outward growth becomes the vagina and the receptaculum seminis. Its develop-

[1] See photomicrographs for circular and longitudinal layers; scattered fibers also occur.

[2] This difference of position, however, is a very constant one. The cells from which the ova develop are early marked off by their position and never take part in any other development. The facts are not opposed to the continuity theory; they simply afford no evidence for it.

ment takes place somewhat more slowly than that of the inward proliferation of cells, due, doubtless, to the fact that its function is exercised only relatively late.

The inward "proliferating area gradually extends somewhat toward the median plane and somewhat toward one surface of the proglottid known as the ventral surface. . . . As the inner and ventral end of the proliferating area approaches the inner layer of circular muscles it spreads out into a flattened somewhat disc-like area exactly as if it had encountered resistance to its growth in the original direction and so had begun to spread out in other directions."[1] A little later the posterior portion of the anlage of the ovary becomes the anlage of the vitellarium which develops about the middle part of the oviduct. This origin shows that morphologically the vitellarium is part of the ovary and gives credence to the view expressed for similar cells of other animals (test cells of ascidians, etc.), that the cells are rudimentary eggs.

There are two regions concerning whose method of growth it is especially important to have definite knowledge. These are the primary anlage and the early embryos. Concerning the former there are four possibilities as to the growth of the cells: origin *de novo*, by migration from the surrounding parenchyma, and by cell division direct and indirect. The first of these as Child remarks ('10, p. 112) is "an alternative which most of us would hesitate to accept without proof of the most conclusive character."

Migration of nuclei to this region, if one could show that it occurred, would explain the relative lack of mitoses in the anlagen. It would then be necessary, of course, to seek for mitoses or amitoses in the neck region to determine the method of origin of the cells. But actual evidence of migration is as impossible to obtain as is evidence of amitosis. At best only indirect evidence of it can be secured.

If abundant divisions occurred there would be little probability of migration, although it might occur. Abundant divisions are not to be seen, however. On the other hand, the fact that many of the nuclei in the primary anlage are smaller than the paren-

[1] Child, '07*b*, p. 99.

chymal nuclei suggests that they have divided. Still a third line of evidence is obtained by a study of the number of nuclei in given regions before and after the formation of the anlage. To make such a study it is not sufficient to count the nuclei in a single section of each proglottid and compare the counts directly, for the proglottid grows in three dimensions. Either the entire number of nuclei in all the sections to be compared must be counted or the ratio determined by a mathematical calculation based upon counts from different fields of the two sections, measurements of the inner parenchymal ellipses (that part of the parenchyma enclosed by the circular muscle layers) and the number of sections of the proglottid.

Such a calculation has been made and its results are given in the following table. While the problem does not lend itself fully to the rigor of a mathematical treatment, it is believed that the results are approximately accurate, at least sufficiently so for the sake of a comparison. In each case two proglottids from the same chain, one near the head, the other farther back, are compared; by nuclear ratio is meant the ratio of the number of nuclei within the circular muscle layer of the one to that of the other; the volumetric ratio represents the ratio of the volumes of the parenchyma within the muscle layers for the same proglottids.

Case.	Stage of Development.	Nuclear Ratio.	Volumetric Ratio.
A1	Testes formed, ovary anlage separate.	6 : 10	7 : 10
A2	Growth period.		
B1	Ovary anlage just separated.	1 : 2	1 : 5
B2	All organs formed, merely growing.		
C1	Primary anlage stage.	1 : 7	1 : 17
C2	Late growth period.		
D1	Early primary anlage.		
D2	Ovary nearing growth period, testes almost all formed.	2 : 9 : 10	2 : 9 : 11½
D3	All organs formed.		
E1	Early primary anlage.		
E2	Testes formed, ovary in growth period.	1 : 6	1 : 7
F1	Primary anlage stage.		
F2	Testes formed, ovary in early growth period.	1 : 7	1 : 7

Of the six cases here given, believed to be fairly representative, in only one, A1 and A2, have the nuclei increased in greater proportion than the volume, and even here the increase is only slightly greater; in two, D1 and D2, and F1 and F2, the two ratios have kept pace with each other; while in four, B1 and B2, C1 and C2, D1 and D3, and E1 and E2, the nuclear ratio has fallen more or less behind the volumetric. Now evidences of division are no more—and no less—common in the parenchyma cells than in the anlage so the result of these four cases favors somewhat the notion of migration of the parenchyma nuclei to the anlage for there are relatively fewer nuclei in the older proglottids and it is hardly probable that degeneration, the opposing factor, would play a part in the decrease in the number of nuclei in as young proglottids as these are. But on the whole the evidence for migration is not strong; certainly this factor cannot have been the important one in the formation of the anlage of the female organs.

It would seem, then, that here as is general throughout the animal kingdom germ cells must arise by cell division. It is the inconspicuousness of these processes that occasions the present discussion.

Mitosis unquestionably occurs. Child records and figures occasional cases. Although he holds that they are too infrequent to account for the cell proliferation he finds them in nearly every stage of development. He has, however, never found them in the early stage of the duct formation, in the primary anlage, unless his recent account of a single individual consisting of scolex, neck region, and a few of the youngest proglottids in which nearly every nucleus is dividing mitotically may include such cases. He has given no definite statement of the stages included in this worm, but certainly the primary anlage appears in the proglottids which are quite young. In these earliest stages I have found a considerable number of mitoses although they are by no means so abundant as to be seen upon a cursory examination. Let me call attention to the fact that in almost any tissue the ratio of resting to dividing cells will be found to be very great. This ratio is a surprising one.

In the oögenesis of *Planorbis*, a form in which divisions are

of the usual mitotic type is shown in a research, as yet unpublished, by Conklin, the division figures are very difficult to find. One may examine section after section without seeing a single case of mitosis. Although I have not made a statistical study of the two cases I am of the opinion, from the examination of the slides which Professor Conklin has kindly shown me, that division figures are more rare in the oögonia of *Planorbis* than in those of *Moniezia*. The ratio of resting cells to dividing cells is large. Yet there is no evidence for the occurrence of amitosis here. Another better known example is found in the oögonia of *Ascaris*. Any one who has searched for oögonial divisions in this animal knows how few cells show them in proportion to the number present.

As development progresses mitoses occur as stated up to the growth period, but, it is true, not as frequently as one would expect from conditions in other tissues. I do not find that the figures are always as easily recognizable as are mitoses in many tissues.

That mitoses occur throughout the development of the female germs cells in considerable numbers, there is not the slightest doubt. The evidence for this is positive. Furthermore, they always occur in the peripheral, *i. e.*, the growing portion of the anlage, a very significant fact. It requires merely a little careful search upon properly fixed and stained material to discover them. Tissues fixed in King's fluid and stained with the Flemming triple stain are especially favorable for finding the mitotic figures. They do not occur as frequently as one would *a priori* expect, but, in the light of the fact which will appear immediately, *i. e.*, that there is no good evidence for the occurrence of amitosis, we are merely forced to readjust our ideas of the number of divisions necessary to produce a tissue. The mitotic frequency has not been sufficiently considered. I regard it as also highly probable that periodicity of division is an important factor in tissue production.

There are to be seen here many cases of nuclei which lie close beside one another with only a narrow layer of cytoplasm between them. There have been some few cases in which no layer could be distinguished, but the character of the material is such

that this easily lies within the limits of observational error. These cases may be considered as evidences of recent division; they are not, however, distinctive of either direct or indirect division. If there were abundant evidence of the former they would be regarded as adding to it. On the other hand, if spindles were very numerous this arrangement in pairs would be looked upon as the final stage in the division of the cells by mitosis. Cases of juxtaposition of nuclei are seen in Figs. 2, 12 and 17.

One may occasionally see in some sections a few nuclei darker than their neighbors (Fig. 18) scattered among the pre-oögonia. They have no visible significance; they differ from other nuclei only in being darker. I have never seen one half of a nucleus darker than the other. I may here add that there is never a dark body, a "Nebendotter" occupying a portion of an ovum as in *Tænia*.

Rarely, indeed, does one find constricted nuclei in my preparations. Shrunken and irregular nuclei—artifacts—occur; strands of linin across a nucleus often resemble the formation of a plate (as in Figs. 41 and 42); the edges of adjacent nuclei overlap and sometimes the overlapping parts are so thin that the true condition is not at first apparent; but the various stages of plate formation, constriction, etc., and especially of "endogenous" division as set forth by Child I have never seen. One does, however, occasionally find a dumb-bell-shaped nucleus; but so infrequent are they that I am unable to determine any significance for them.

I find myself, therefore, unable to confirm Child's conclusions for the various reasons given. His position, however, that the "relative frequency of mitosis and amitosis in certain species, and even in single individuals may vary greatly according to conditions" ('10, p. 116) is an unassailable one with our present data.

The Oögonial Period.—From the disc-like anlage on the ventral side of the proglottid upward projections or follicles develop and give to the ovary its characteristic form.[1] About the time of full development an enveloping membrane appears probably as a differentiation of the surrounding parenchyma. For the female

[1]Cf. Photomicrograph III.

germ cells from the differentiation of the ovarian anlage to the formation of the membrane of the ovary the term "pre-oögonia" has been (aptly, I think) suggested by Professor Dahlgren (see Figs. 9–14). It is only at the end of the pre-oögonial period that the cells which are to form ova are certainly distinguishable from all others. The convenience of the use of this term will be apparent. The formation of the follicular membrane seems to mark the time of cessation of active proliferation on the part of the oögonia. I have seen no sign whatever of cell division in stages following this. There are no longer many parenchymal elements to be seen in the ovary and each oögonium becomes sharply marked off from its neighbors. The subsequent growth of the ovary is merely that attendant upon the growth period of the oögonia. The cell divisions are, of course, limited to the maturation and cleavage divisions.

The Growth Period and Behavior of the Nucleolus.—The growth period in the cestode as in most animals is relatively long; many more proglottids have the ova in this condition than in any other previous to the cleavage stages. The actual duration of the growth period is probably three or four months.[1] It is well known that sheep older than yearlings are rarely infested with tape-worms. The parasites occur only in the young, but nevertheless they attain a length of 4–5 meters in the case of *M. expansa* and of 2 meters in the case of *M. planissima*. Developing embryos first occur at a distance of 100–110 cm. from the head; the primary anlage appears 7–8 cm. from the head; and the growth period of the oögonia begins about 20 cm. from the head. This short duration of the stage in which multiplication of the oögonia is taking place must be kept in mind when investigating the method of cell division here. To Child's description of the growth period little can be added. Briefly, there is accomplished during the period the great growth of the eggs, synapsis, the formation of the yolk globules, and certain contemporary changes in the nucleolus to be described immediately. During this period of no divisions the oögonia after synapsis are characterized by a minimum amount of chromatin, faintly staining, distributed peripherally, although strands may reach the nucleolus.

[1] These time limits are tentative for I have been able to approximate them, only

Concerning the structure of the nucleolus more can be seen during the growth period than at any other stage of development. In favorable preparations, the nucleolus in oögonia, oöcytes and segmentation cells shows a peripheral portion staining violet with the triple stain, and a central portion, the so-called vacuole, staining with safranin. During the growth period additional details appear. These are (Figs. 28–30), within the vacuole, a small more or less refractive body, the endonucleolus (cf. Montgomery, '99), staining very dark with gentian violet, a reticulum supporting this body, and in the outer peripheral portion of the nucleolus certain darker bodies. Certain appearances here very much resemble the conditions described in the karyosome cycle of *Diplodiscus* by Cary ('09). There is, however, no parallel in the deve opment.

Only one true nucleolus is present in a cell. Besides this there are frequently one or more chromatin bodies, karyosomes, which with iron hæmatoxylin stain like nucleoli. But the gentian violet or Ehrlich-Biondi preparations never show more than a single true nucleolus.

As to the nature of the true nucleolus there is not much evidence. Young deplores the use of the term "nucleolus" and calls the structure a "nuclear granule." It is from the nuclear granules, he holds, that the nuclei develop; the granules in turn arising *de novo* (probably) from a common "cytoblastema." "Containing, as they do in many cases, most of the staining matter of the nucleus, they represent rather the chromatin than the true nucleoli." I am obliged to dissent from this view; they are without doubt true nucleoli, as differential staining shows. Nevertheless, in addition to their nucleolar character they may well serve as chromatin reservoirs during the resting period of the nucleus. Indeed, the peripheral portion stains with gentian violet just as does chromatin, and the darker bodies easily suggest chromatin masses. The fact that the nucleoli increase in size like the other elements of the cell during synapsis is explained by the swelling of the vacuole, for this occurs markedly; in many cases several vacuoles are present. Furthermore, the nucleoli are always in intimate connection with the spireme, and of course they disappear when mitosis comes on. C. v. Janicki ('03) finds

that in the trematode, *Gyrodactylus*, the chromatin goes into the nucleolus during the resting stage.

Synapsis.—Whether or not chromatin actually comes from the nucleolus, the nucleus in the early part of the growth period acquires an abundance of chromatin and a spireme stage at once ensues. The spireme, in contact with the nucleolus, develops rapidly and becomes massed on one side of the nucleus just as in the "bouquet" or "synapsis" stage of the first maturation prophase. Although some time intervenes between this and the prophase of the first oöcytic division, I regard this as probably a true synapsis. What seems to be conjugation of the chromosomal loops occurs (Fig. 26) and all the characteristics of a synapsis are present. Furthermore, precocious synapses are not unknown (*e. g.*, see Montgomery on *Euchistus*, '01). Beginning in the medullary portion of the ovary, synapsis spreads rapidly over the entire organ, involving nearly all the oögonia at once, although they are in different stages in different parts. At the same time the cytoplasm is increasing in amount but there is no evidence of a causal relation between the two phenomena except that they are synchronous. The nucleus is as a rule excentrically placed and the "bouquet" is frequently found on the side of the nucleus next to the greatest cytoplasmic mass, but there are many exceptions to this arrangement. Following the "bouquet" stage the spireme spreads throughout the nucleus, loses its property of staining very densely and takes on the appearance of the typical resting nucleus about to undergo maturation. It remains in this stage for a relatively long period during which yolk production is accomplished and the ovum attains its full size.

Yolk Production.—Yolk production rarely begins before the "bouquet" stage has passed off. Usually upon the same side of the nucleus as that where the "bouquet" occurred, but always in a part where the cytoplasm is abundant, an area staining more deeply than the rest of the cytoplasm is visible. "It is comparable to certain of the differentiations which have been called yolk nuclei in other eggs and its appearance is followed almost immediately by the formation of yolk granules which are contained in the egg cell itself" (Child). Often two or more of these yolk-producing areas are to be seen in the same ovum.

In certain favorable material I can see a central dark granule about which appears a clear brownish court (iron hæmatoxylin material) and outside of this the yolk granules develop (Figs. 31 and 32). Whether this is general I am unable to decide; probably, it is. Child thinks, and it seems a logical conclusion, that the bouquet stage is responsible in some way for the yolk production. Similar conditions have been described by Janicki for *Tænia serrata*, and by several authors, particularly Goldschmidt, for trematodes. Goldschmidt[1] thinks that during the spireme stage a separation of somatic and germinal substance occurs and that the "trophochromatin" escapes from the nucleus to reappear later in the cytoplasm as yolk nuclei. Somewhat analogous accounts have been given for the eggs of animals of other groups.[2]

The yolk of *Moniezia* differs from that of *Tænia* in that in the former it occurs as spherules filling the entire cytoplasm while in the latter there is only a single mass (in the earlier stages more) as large or larger than the nucleus lying in the cytoplasm.

The Ovarian Egg.—The end result of these processes is the ovarian egg. It is irregular in shape due to crowding by surrounding cells and neighboring eggs, and contains a large germinal vesicle located asymmetrically. Filling the cytoplasm more or less completely are yolk globules of various sizes. The nucleolus is now comparatively small and is located near the periphery of the nucleus. Chromatin is distributed throughout the nucleus in the form of fine granules attached to a linin reticulum. I cannot say that centrosomes and centrospheres occur but I have seen some structures resembling them (Fig. 34). In this condition the egg is ready for passage through the oviduct where fertilization occurs. The lumen of the oviduct where it enters the ovary is much smaller than the diameter of the egg so that pressure is exerted on the egg during its passage causing it to change its shape. After reaching the uterus it regains its spherical form.

The Vitellarium.—Early in the development of the anlage of the female sex organs there is set aside a certain portion of the

[1] See Janicki, p. 695.

[2] Cf. Conklin on *Crepidula*, '02.

cells to form the vitellarium and shell gland. It lies posterior to the part which becomes the ovary arising from a group of cells branched off from the middle portion of the anlage—that portion which becomes the oviduct. The medullary portion develops first becoming the shell gland; from the periphery of this newly differentiated anlage are proliferated cells which become the vitellarium proper.

The morphological relations of this organ and the ovary suggest that the vitellaria are fundamentally ova specialized along another line than reproduction. This view, I think, is clearly supported by the evidence.[1] I have, therefore, endeavored to find a parallel in their development; it is not, however, a very close one. They agree in that they early pass through their division stages and do not proliferate during the production of yolk. (I have seen only a single case of division, and that not clear, in a nucleus of that period.) They differ in that the oögonial nuclei continue to increase in size long after the vitellarium nuclei have reached their full growth and the former are always more chromatic than the latter. The vitellaria never go through the synapsis stage and the method of yolk formation is unlike that of the oögonia. Small spherules which fuse with one another are formed in the cytoplasm. The mass thus arising grows larger pushing the nucleus to one side. The completed cell looks not unlike a fat cell of the "signet ring" type.

Like the ovary the vitellarium at the time when cell multiplication stops develops a membrane separating it from the other organs of the complex. Previous to this stage the cytoplasmic boundaries have not been as clearly defined as those of the other cells of the primary anlage, but now they become quite distinct and the cells more dense. The nuclei develop more chromatin, not in the form of a spireme as in the case of the ova, but as enlarged granules giving to the nucleus the appearance of several nucleoli. Sometimes strands may be seen extending from the nucleoli out to the periphery where the yolk masses are forming.

The further history of the cells of the vitellarium is of great interest from the standpoint of the histology of secretion but is outside the scope of the present problem.

[1] Cf. Child, '07*b*, p. 113.

Although the vitellarium and the ovary arise from the same anlage and at first are distinguishable only by their position they soon acquire histological differences. The nuclei of the former contain very lightly staining chromatin in the form of a reticulum but there is a large nucleolus. It frequently occurs that there are two or more nucleolar-like structures, karyosomes, in a nucleus. The appearance of the nucleus itself is that of an almost empty court surrounding the nucleolus or nucleoli. The nuclei of the oögonia, on the other hand, do contain a reticulum, rather lightly staining, it is true, but consistently present. While the cytoplasm is more indefinite in amount in the vitellarium it can be seen that the ratio of nucleus to cytoplasm, if the inclusions are not taken into consideration, remains much more constant; if the yolk mass be considered the second term of the ratio is much increased. At no time after they become clearly distinguishable are the cells as large as the oögonia, and at maturation the oöcytes are more than twice their size.

As to cell multiplication I am convinced that after a comparatively early period division ceases. During this early period clear cases of mitosis are to be seen, but, it is true, as Child says, less frequently than in the ovary. On the other hand, the arrangement of nuclei in pairs is, perhaps, more in evidence here and indented nuclei are somewhat more numerous. The indentations do not, however, give a series of autoconstrictions from slight indentation up to complete division. If mitosis is not clearly proved as the sufficient cause of cell multiplication amitosis is certainly less so; for there is positive evidence that some mitoses do occur, but for amitosis there is only negative evidence.

The Female Genital Ducts.—From the same anlage which gives rise to the ovary and the vitellarium, the female[1] genital ducts develop. Therefore, although they are strictly speaking somatic structures, their cells will be considered here, with regard to the method of cell division, as derivatives of the female anlage. Their early history, therefore, is the same as that of the ovary and vitellarium. A thickening of nuclei appears median to the longitudinal excretory tubes and gradually extends itself both

[1] The development of the male ducts will not be touched upon here.

outward and inward. The outward extension becomes the vagina, seminal receptacle and fertilization canal; the inner not only gives rise to the ovary and vitellarium but also to the remaining part of the oviduct, the vitello duct and the uterus. These various structures are in no sense an invagination from the outside for their development begins in the middle part of their length and the connection with the outside, through the cirrus pouch, is quite secondary to the formation of the lumen of the tube. The lining is, of course, an epithelium; but it is not an invagination from the exterior. That of the cirrus pouch, however, is an invagination and might be looked upon as an ectoderm. But in the other parts of the sex ducts the epithelium is clearly not ectodermic in origin although histologically it resembles the cuticle and is connected with it through the cirrus pouch.

In their histological structure, the walls of the uterus, oviduct and seminal receptacle are made up of a simple layer of flattened cells probably with a basement membrane. The oviduct furnishes exception to this statement at its opening into the ovary where a layer of circular muscle fibers is demonstrable, and in that part of its course which is known as the fertilization canal. In this latter canal the structure more nearly resembles that of the vagina. The vagina beginning at the lumen consists of ciliary projections, a layer of nuclei embedded in a homogeneous cuticle, longitudinal and circular muscle fibers and a cellular layer.

These canals develop fairly quickly and have all (uterus excepted) finished their growth as far as cell division is concerned long before the sex products call for their use. Upon the passage of the eggs from the ovary they degenerate and their place is taken by the embryo filled uterus.

Whether the ducts grow by more and more parenchymal nuclei becoming involved in the proliferating area, as Child thinks, rather than by the actual outgrowth of the original anlage by the multiplication of its cells is not clear to me. It seems probable that the extension occurs by both methods of growth. The oviduct and the vitello duct are unquestionably formed by the differentiation of part of the cells in the primary anlage for it is

in the middle of the anlage that these ducts develop. In the vagina it is not certain that the parenchyma cells whose position it occupies were numerous enough to provide all the cells in its walls without the aid of some outward growth from the region already formed. There is no fundamental difference, however, in the two methods for in either case the cells are of parenchymal origin.

The conditions in the early primary anlage have already been set forth. Mitoses are few, but they do occur. There is no certain evidence for amitosis although many nuclei are arranged in pairs. I have previously given my reasons for the view that amitosis does not occur at this stage of development.

But in the stages beginning with those in which the anlagen of the various organs can be distinguished the method of cell division can be conjectured only. Mitoses are very few, indeed, and occur only in the borders of the proliferating region. On the other hand, the nuclei are small and irregular in size and are frequently closely crowded together in groups of two, three or even more. My preparations do not show actual cases of amitosis, however, although conditions would seem to be quite favorable for its occurrence. Other than the rare mitotic figures which occur there is absolutely no evidence as to the method of nuclear multiplication (see Figs. 51 and 52).

The opinions with regard to the regions in which active cell divisions are occurring expressed by Child ('07*e*) in the following statements do not seem to be well founded according to my observations. He says: "In the early stages there is a marked difference in the size of the nuclei in the central and the peripheral portions of the area in which proliferation is occurring. . . . Evidently proliferation is much more active in the central than in the peripheral regions of the proliferating area. In somewhat later stages the rapidity of division apparently decreases and the nuclei of the central regions gradually increase in size until they are almost or quite as large as those about the periphery. . . . From this stage on the differentiation of the walls of the ducts gradually takes place: muscle fibers develop, a lumen appears, and nuclear divisions become less and less frequent."

In this quotation (the omitted parts have to do only with

details not bearing upon the point which I wish to consider) the criterion of proliferation seems to be smallness of nuclei, although in the figures illustrating these statements constricted nuclei, amitoses, are present. As previously stated, these regions in my sections do not show amitoses. But mitoses are to be seen (rarely, it is true) in the borders of the proliferating region. The presence of mitotic figures in this region, it seems to the present writer, is a most significant fact—where but in the borders of a region which is extending its area should division figures be found?—and the small size of the nuclei at the center is of little importance. These central cells are merely beginning differentiation while the proliferation takes place at the periphery of the sex duct anlage.

The method of cell multiplication in the female sex ducts of *Moniezia* cannot at the present time be positively stated. I find no certain evidence for amitosis and that for mitosis is, perhaps, insufficient to account for the growth which has taken place. Nevertheless, I have seen some cases of mitosis here.

Fertilization and Maturation.—The process of fertilization in the eggs of *Moniezia* can be followed only with the greatest difficulty. Although one may examine carefully a great number of proglottids he will rarely find a stage showing the entrance of the sperm and its course prior to the maturation divisions. I am, therefore, unable to amplify the account given by Child ('07*e*) of the early stages of fertilization.

From the ovary the passage through the oviduct to the uterus is so rapid that eggs are seldom found in that part of the duct. Linton ('08) has observed the process of fertilization in a live trematode, and found it to be of very short duration (less than 40 seconds). In *Moniezia* one often finds segments with embryos in the uterus undergoing maturation and eggs in the oviduct ready for fertilization, but the cases are rare in which there are eggs in the fertilization duct. Doubtless here also the passage is very rapid and probably, as Child thinks, periodical.

The eggs pass from the ovary into the uterus through a more or less coiled oviduct. They meet the spermatozoa at the mouth of that branch of the oviduct which comes from the seminal receptacle and are there fertilized. Near this point the vitello

duct after passing through the vitellarium and shell gland joins the oviduct—the point of union being known as the oötyp—and this latter duct continues to the uterus.

Cases of polyspermy frequently occur; that is, some eggs show more than one male pronucleus. I have not observed the entrance of several spermatozoa into the same egg, but this is not surprising since the entrance of a single spermatozoön is rarely seen. In many proglottids some of the segmenting eggs ultimately degenerate; possibly they include the cases of polyspermy.

In certain proglottids ova are found in the seminal receptacle, sometimes in quite large numbers. As the seminal receptacle is not ruptured in many of these cases it would seem that in passing through the oötyp the eggs had crowded past the branch of the oviduct leading to the uterus and forced their way through the fertilization canal into the seminal receptacle. There they degenerate. It is, of course, very probable that polyspermy occurs here but so densely packed are the spermatozoa about the egg that the process cannot be followed.

For figures of the fertilization stages as well as for additional figures of the maturation divisions the reader is referred to Child's paper ('07*e*).

Child's account of the maturation divisions agrees in essentials with my observations, and I shall, therefore, merely give a brief résumé of this stage of development.

The entrance of the spermatozoön furnishes the stimulus for the formation of the vitelline membrane and for the beginning of the maturation divisions. The first polar body is usually formed by the time the egg has entered the first loops of the uterus and the second polar body follows soon after. The first division requires more time than the second if the relative frequency in which the two stages occur may be regarded as a criterion. Many more first divisions occur in my material than second.

The characteristics of these divisions are large globular centrosomes—ring-shaped in section—faint astral radiations,[1] and a very long spindle on which small chromosomes are to be seen

[1] Child stated that he was unable to see asters but thought they probably occur. I have succeeded in distinguishing faint radiations in numerous maturation stages.

distinctly. An equatorial plate is not usually formed as the chromosomes pass to the poles irregularly. Figs. 53 and 54 illustrate these facts.

The reconstruction of the female pronucleus is shown in Fig. 55. These stages suggest amitotic division of the ovum—some more than that figured; their true nature, however, is readily ascertained.

I am unable to see that the maturation of *Moniezia* has any bearing whatever on the "hypothesis of individuality" of chromosomes, which Child says these mitoses do not appear to strengthen, for it does not seem to me that evidence either pro or con is presented here. Indeed, the whole question involved is not so much whether the chromosomes maintain their individuality as it is whether the mechanism of division will give an exact sorting of the male and female elements in the germ cells or a mere separation of unequal parts of the nuclei. If mitosis could be established as the universal method of cell division that fact alone would by no means warrant the assumption of chromosome continuity, as Child would seem to imply that it would do. Among those who believe that mitosis will be found general in germ cells are many who do not accept the individuality hypothesis as it is now put forward. Furthermore, proof of that hypothesis will demand observations upon a more favorable object than *Moniezia*. I would emphasize the fact, therefore, that *Moniezia* presents no evidence upon the individuality hypothesis.

Cleavage.—The steps of the cleavage process in *Moniezia* were discussed in 1881 by Moniez from an embryological point of view, and by Child in 1907 from the point of view of cell division. The results of Moniez are very suggestive and deserve to be repeated in the light of more recent discoveries on cleavage and with later methods of technique. Since the cleavage of *Moniezia* is deserving of this broader treatment I shall not here attempt other than the most general statement of the process but shall concern myself with the method by which segmentation is accomplished.

The type of cleavage, as will be seen from a glance at the figures, from 56 on, is that of a large macromere giving off small

micromeres at the animal pole.[1] The later divisions bring about the formation of a morula which gives off two layers by delamination and then develops into the hexicanth embryo. This cleavage especially in its earlier course differs markedly from that in *Tænia serrata* (cf. Janicki's figures with mine).

As cleavage proceeds many embryos become elongated, a condition which Child suggests is due to pressure of the uterine walls. This, of course, does not represent the future axis of elongation. That Child's suggestion is probably the correct one is shown by Moniez's figures from entire embryos which are all spherical and do not include any elongated stages.

During the early part of my study while I was working upon material in which the results of fixation were good but by no means perfect I thought I had found the syncytial condition of which Child speaks: "In most cases a number of nuclear divisions occur before cell boundaries become visible in the egg. . . . As cleavage proceeds the egg is gradually divided into blastomeres containing yolk and blastomeres without yolk. In earlier stages the yolk bearing blastomeres often contain two or more nuclei, but in the later stages after cytoplasmic cleavage is more advanced they usually contain one relatively large nucleus. In other words as these yolk bearing blastomeres are gradually reduced in size by successive cleavages the cytoplasmic cleavages keep pace more nearly with the nuclear divisions. In the yolkless portion of the egg, however, nuclear division continues far in advance of cytoplasmic division as far as the cleavage has been followed; the consequence is that each blastomere contains several or many nuclei of relatively small size."

Later, in studying material in which fixation had been more nearly perfect, I saw at once that the supposed syncytial condition was in reality an artifact. From the very first the micromeres can be distinguished as such, an observation corresponding with the figures of Moniez made about thirty years ago. The cytoplasmic membrane becomes completely constricted even before the telophase is finished as in Fig. 57. In properly fixed material I have never seen an egg syncytium. The work of

[1] Assuming that, as in all known cases except in the ctenophores according to Korschelt and Heider, the polar bodies indicate the animal pole.

Moniez contains much which a reinvestigation with modern methods of technique will substantiate.

As regards the method by which segmenting eggs divide, the following sentences are illustrative of Child's position:

"But although the first cleavage is usually or always mitotic, there can be no doubt that amitotic division appears very early in the course of cleavage.

"Cases of mitosis are rarely seen after the first cleavage but amitosis is of frequent occurrence.

"Rarely a case of mitosis is observed: in all the hundreds of eggs in cleavage stages which have been examined, not more than a dozen cases of mitosis have been seen in stages later than the first cleavage. When mitosis occurs it apparently always involves one of the larger nuclei. Mitotic divisions of the small nuclei in stages like those shown in Plate VI. have never been observed. The smaller nuclei are, without doubt, dividing more rapidly than the larger, and we are probably justified in concluding that amitosis occurs in those regions of the egg where division is most rapid, while mitosis is found, when it occurs at all, among the nuclei which are dividing more slowly."

With the first of these statements only can I agree. Figs. 56 to 63 show the successive steps in the cleavage process up to the eight cell stage and every division is by mitosis. Fig. 64 shows several cells from an embryo of thirty cells, among which are two blastomeres in mitosis. Fig. 65 is drawn from a single blastomere in an embryo of from fifty-five to sixty cells; it shows an early phase of mitosis. These stages are not rare or difficult to find in my material. I have examined segmenting eggs of numerous other animals and do not hesitate to say that the mitotic figures in *Moniezia* are as frequent as in other lots of eggs selected at random.[1] Indeed, in five sections cut four micra in thickness, in which the uterus did not contain a relatively large number of eggs over twenty cases of cleavage mitoses which were deemed worthy of sketching were found. In another case, in 125 embryos (all that were present in the given region)

[1] Of course in those cases where artificial fertilization is practical the eggs are selected in the desired stage; such cases may not fairly be compared statistically with the one under discussion.

there were 28 cleavage mitoses; in another of 211 embryos were 53 mitoses; in still another, 124 embryos showed 40 mitoses. The evidence that these cleavages occur by mitosis is of the most positive and convincing character. Cleavage mitoses are more abundant in my material than the second maturation division which is, of course, mitotic.

Furthermore, I have seen only one condition which could possibly be interpreted as amitosis; it is shown in Fig. 59. This figure represents the stage following the telophase of the division cutting off the third micromere. The same condition obtains following other telophases, however. The nucleus after the telophase at first takes on an irregular appearance but later becomes rounded out into the typical shape. This stage is exactly comparable to the reconstruction stage of the female pronuculeus after maturation. It is to be interpreted as the final phase of a mitosis rather than the precursor of an amitotic division.

In the later cleavages, although I have not as yet followed out all of the cell lineage, I have never found any reason to doubt that mitosis is the method of cell division. Mitosis certainly occurs frequently and I have seen no evidence of amitosis. The two figures given are of typical cases; further illustrations would be mere reduplication of the evidence.

In the smaller blastomeres I have never seen any sign of direct division; on the other hand, Figs. 60 and 62 show cases of mitosis in these cells.

To the present writer the facts in the cleavage of *Moniezia* seem to admit of no other interpretation than that mitosis is the method by which segmentation is accomplished.

Summary of the Evidence.

This summary will include the evidence gained from the study of the female sex products from the time of their appearance as the primary anlage of the ovary to the completion of segmentation. It will not include the vitellarium of sex ducts since the evidence given by them is not thought sufficient to be conclusive in favor of either view.

It has been stated that the cells composing the primary anlage

might arise *de novo*, by migration, by amitosis and by mitosis. The first of these possibilities was dismissed as highly improbable; the burden of proof is upon him who upholds this view. The second method of origin was admitted to be possible, but it was thought to play only an unimportant rôle, if any at all, in the development; and even if the assumption of migration as a factor—positive proof would scarcely be procurable—were found to be necessary it would merely throw the question of division back into the earlier stages. It was held, therefore, that the cells under discussion must arise *in situ* by direct or indirect division. It is the purpose of this summary to present the evidence for the two views as concisely as may be.

It has been shown, furthermore, that at two periods of development only may active growth by cell division be looked for: namely, during the period of pre-oögonial and oögonial divisions and during the cleavage of the embryo; the rest of the growth involved is that of increase in size and in differentiation. The discussion, therefore, limits itself to the question: is amitosis or mitosis the method by which the pre-oögonial and oögonial and the cleavage divisions occur?

The evidence, as I have found it, favoring the view that amitosis occurs during the periods of development mentioned is as follows:

First, there are in the development of the oögonia fewer cases of mitosis to be found than one would *a priori* expect, and it might be claimed that the number present is not sufficient to account for the cell multiplication which must have occurred.

Second, the arrangement of the nuclei in pairs suggests in the light of the fact just stated that the cells may have arisen by direct division. Two nuclei often occur close together but separated more or less from their neighbors.

Third, constricted and indented nuclei do occur in the early stages of development of the oögonia; but they occur in my material only in cases of imperfect fixation, so they lend very little support to the amitosis view.

Fourth, in the cleavage stages but one condition (unless accessory sperm nuclei might be confused with direct cell division) has been seen in properly fixed material which might be inter-

preted as favoring the amitosis view: the condition represented in Fig. 59.

The evidence opposed to amitosis and favoring the view that mitosis is the method by which divisions occur is as follows:

First, mitotic figures do occur throughout all stages of the development of the tissues considered, and they occur in the peripheral (or growing) regions of the organs. Analogy may be drawn from cases like *Planorbis* and *Ascaris* in which the oögonial divisions are very difficult to find, and yet are known to occur regularly.

Second, stages of nuclear constriction are not found in sufficient number to account for the arrangement of the nuclei in pairs if amitosis be the method by which they arise. It is logical to expect that steps in the process would be found if the arrangement in pairs is the end result of amitotic division. As has been repeatedly stated, it is not possible to establish a complete series of stages in the auto-constriction of nucleus and cell body. If periodicity is a factor in cell division (see below) the arrangement may be looked upon as favoring the mitosis view. Finally, it is most probable, although impossible to prove, for the preoögonia and oögonia are not favorable for such study, that there may be some movements of the halves toward each other analogous to the movements of telokinesis in other tissues, for in all cases carefully studied with regard to this point, including developing eggs, epithelial tissues, etc., such movements have been found. I have myself found evidences of them (not shown in the present series of figures) in the cleavage of *Moniezia*.

Third, the occasional cases of constriction and indentation which occur have not been shown to be normal steps in division.

Fourth, the condition represented in Fig. 59 is, as previously stated, the final stage of mitotic division rather than the beginning of amitosis.

Fifth, the evidence favors periodicity as a factor in mitotic divisions. (See below, under discussion.)

Sixth, that the maturation and first cleavage alone should occur by mitosis as described by Child is difficult to reconcile with amitotic divisions elsewhere throughout the series.

Seventh, the evidence that the cleavage divisions occur by

mitosis is of the most positive character. It cannot be too strongly emphasized.

Eighth, I have after diligent search upon carefully prepared material been unable to establish a series of stages in the autoconstriction and subsequent division of the nucleus and cell body by amitosis.

Considering the evidence as set forth, it seems to the writer that one is forced to the conclusion that mitosis is the method by which pre-oögonial, oögonial and cleavage divisions are accomplished. These are the divisions to which the chief interest attaches.

Discussion.

Since the account of Child appeared describing amitosis in *Moniezia* and other forms there have been a number of *a priori* suggestions offered by various workers as possible explanations of the conditions which he describes. It will be of interest to examine some of these suggestions in the light of the facts which I have observed in *Moniezia*.

Cary on the basis of his observations on *Diplodiscus* suggests that the telophase of an intranuclear mitosis would give all the appearances of true amitoses. I have carefully searched for evidences of intranuclear mitoses in *Moniezia* but have found none. I have seen cases which at first resembled a faint intranuclear spindle, but upon more careful study it became clear that they were merely chance arrangements of linin threads and chromatin granules and not mitosis. Furthermore, as already shown, distinct cases of mitosis of the regular type are not difficult to find. It is, of course, true that the condition which Cary describes would resemble amitosis, but I have not found it present in *Moniezia*.

Boveri calls attention to the fact that figures similar to those found in *Amblystoma* (Child, 07*a*) are also found in *Triton*. In this latter form Rubaschin found that after mitosis the nuclear material does not fuse to form a single vesicle in the blastomeres but two are always formed. They never separate but go through mitosis together. In *Moniezia* there is no evidence of this kind of phenomenon.

As there is no "Nebendotter" in the eggs of *Moniezia* my

former suggestion that that structure might give a misleading appearance of amitosis is not borne out.

As polyspermy occurs here accessory sperm nuclei might be wrongly interpreted as amitosis. The condition, however, is not difficult to distinguish.

Cell migration has already been discussed in detail as a method of origin for the pre-oögonia. As stated before there is not much evidence that it occurs. It certainly is not the exclusive factor and probably not even an important one.

There yet remains my suggestion that mitosis may be of short duration and occur in waves or at more or less definite periods depending upon some unknown physiological factor—an interpetation which Child refuses to accept. He says ('10, p. 113): "Not only the cells of the neck region but the cells of the scolex, which does not take part in the growth of other regions are undergoing mitosis in this specimen.[1] Moreover, I am as yet unable to convince myself that the many cases of apparent amitosis which I have observed in the neck region of other individuals, are errors of observation, or something else than nuclear division." Nevertheless, is not the position that "the relative frequency of mitosis and amitosis in certain species and even in single individuals may vary greatly according to conditions" in its practical application an admission of my suggestion?

This suggestion is based first upon certain *a priori* considerations. Beckwith described cleavage mitoses in *Pennaria* and *Clava* occurring from 4 to 6 A.M. only; in certain insects and many plants mitosis occurs at night only; in onion root-tips there are two periods of mitotic activity daily, at 1 P.M. and at 11 P.M. and if the specimens are collected at any other times than these there will be found a paucity of division figures. I have seen grass root-tips in which not a mitotic figure could be seen although young cells of the proper age for multiplication were abundant. Amitosis has never been suggested for such cases as this. Secondly, the suggestion has support in certain

[1] A young specimen, found subsequent to his earlier accounts, consisting of scolex, neck region and a few of the youngest proglottids in which almost every nucleus is undergoing mitosis.

observational evidence not the least of which is Child's young worm in which almost every nucleus is in mitotic division. The fact that I am unable to convince myself that amitosis occurs and the fact that mitosis does occur in some specimens in greater abundance than in others (this applies to *Tænia* as well as to *Moniezia*) and even some organ systems show more figures than others (I have one specimen in which the majority of the testes cells are in mitosis) point strongly to periodicity as a factor in growth. The arrangement of nuclei in pairs may be regarded as a point in favor of this view. That the scolex of this worm should be in division is of no significance for even if it takes no part with the neck region in the formation of new proglottids, and this is not demonstrated, its own growth must be accounted for. The scolex of a young worm is smaller than that of an older one.

Furthermore, if it can be shown that the eggs pass through any one stage periodically it becomes all the more probable that they also pass through others periodically. The evidence is strong that the eggs undergo maturation in this manner, and that fact is most easily explained by periodicity of oögonial divisions.

Child holds that mitoses decrease in frequency with increasing age. Let me suggest that perhaps that factor, whatever it may be, that operates to free the intestine of adult sheep from tapeworms may also operate to check mitotic division during the latter part of the period in which the worms can yet live there.

The evidence, I believe for the various reasons given, strongly favors periodic mitoses as an important factor in the growth of the cestode, *Moniezia*.

Comparison of *Tænia* and *Moniezia*.

Throughout the course of this discussion it has been made clear that *Moniezia* differs from *Taenia serrata* in numerous details, both anatomical and cytological. Yet it is also clear that the differences, while often striking, are differences not of physiological activites and principles but of structural features and structural differentiation.

Most striking of all the differences is that of gross structure

as seen in the female reproductive apparatus. Such wide variations from a common type are not usually found within a single family of animals. In both the uterus is single[1] and median but the other organs are arranged in pairs, there being two ovaries and two sets of auxiliary glands and ducts as well as two genital pores in each proglottid in *Moniezia* while in *Tænia* the ovaries are double but they discharge into a common duct and the other parts of the reproductive organs are single.

But the most interesting comparison of the two forms for present purposes is that regarding cytological characteristics. In both the anlagen of the organs arises as a thickening of the parenchyma cells and the period of cell proliferation is strictly comparable on the two. In *Tænia*, however, it is of less duration as seen by the fact that it extends over fewer proglottids. The method of yolk formation is dissimilar in appearance only, for if the yolk granules of *Moniezia* were to accumulate on one side of the nucleus and fuse the condition would be exactly that of *Tænia*. I have not discovered that there is any difference in the principles of division operative in the two forms.

Between the types of cleavage and early development of the embryos of the two forms there are some noteworthy differences but as they are embryological in character they may not properly be included here. It will be seen from this comparison that there are sufficient differences between *Tænia* and *Moniezia*, although they are members of one family, to justify the claim that observations on the former cannot be used *a priori* as evidence against the latter; but that actual study does not disclose any real differences in the principles of growth and development in the two cases.

Conclusions.

In conclusion, the evidence presented in this investigation goes to show: (1) that it cannot now be positively affirmed whether mitosis or amitosis is the method which obtains in the development of the vitellarium and female genital ducts of *Moniezia;* (2) that in the early stages of sex cell development mitosis unquestionably occurs (probably periodically), while amitosis is not

[1] Stiles and Hassal state that the uterus of *Moniezia* is ontogenetically double.

evident in my preparations; and finally, (3) that there cannot be the slightest doubt that the cleavage of the ovum takes place by mitosis.

NOTE.

While this paper was in press an article by Gough ["A Monograph of the Tapeworm of the Subfamily Avitellininæ, being a revision of the Genus *Stilesia*, and an account of the Histology of *Avitellina centripunctata* (Riv.)"] appeared in the *Quarterly Journal of Microscopical Science* for February, 1911, wherein he describes the development of the eggs of *Avitellina*. He, too, finds Zenker's fluid best for cestodes. As in *Moniezia*, yolk cells do not become attached to the eggs to form compound structures. The large mass which he calls chromatin (Figs. 57–63) apparently correspond to my nucleoli (my Fig. 30); there can be no doubt that in *Moniezia* these are nucleoli and that no other nucleolar structures occur, as shown by differential staining. The extrusion into the cytoplasm of chromatin which breaks up to form the yolk nucleus (see p. 373) has not been observed in *Moniezia*. I would suggest that his Fig. 63 perhaps corresponds to my Fig. 26 which is a synapsis stage not merely the first step in a mitosis. Gough speaks of the maturation mitoses as occurring very rapidly. As *Stilesia*, and, therefore, *Avitellina* is quite closely related to *Moniezia* the comparison of the two is of interest, particularly with regard to the fact that compound eggs do not occur in either.

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Explanation of Plate I.

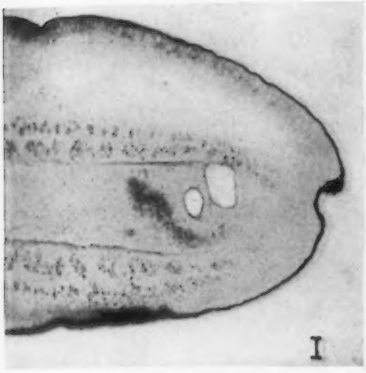
Photomicrographs were made with a Leitz no. 3 objective and no. 2 ocular except IV. in which a no. 1 ocular was used. All figures were drawn with a Zeiss compensating ocular no. 12 and a 1.5 mm. objective at table level with the aid of a camera lucida, giving a magnification of about 3,500 diameters.

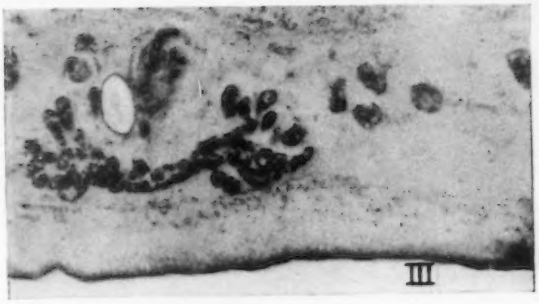
Photo. I. End of a section through a young proglottid, showing the primary anlage of the female reproductive organs.

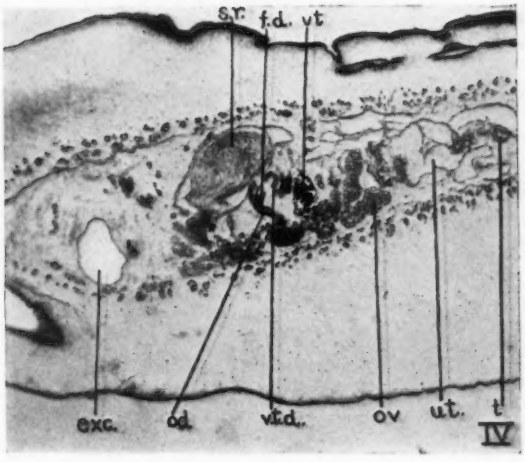
Photo. II. Primary anlage differentiated into the anlagen of the ovary, vitellarium and oviduct.

Photo. III. Later stage showing finger-like processes of the ovary. Dorsal to the middle of the ovary is the tip of the vitellarium.

Photo. IV. Stage of maximum development. *exc*, excretory canal; *od*, oviduct; *v.l*, vitellarium; *vt.d*, vitello duct; *ov*, ovary; *ut*, uterus; *t*, testes; *f.d*, fertilization duct; *s.r*, seminal receptacle.







A. RICHARDS

Explanation of Plate II.

Figs. 1 and 2. Parenchyma cells for comparison with the cells of the primary anlage; from young proglottids. Fig. 2 illustrates the arrangement of the cells in pairs common in all undifferentiated tissue of the cestode. Cytoplasmic boundaries are difficult to make out in such cells as these in all but the best fixed specimens.

Figs. 3–5. From primary anlage in same section as Fig. 2. Fig. 3 is from the inner growing end of the anlage, to be the ovary; Fig. 4 is from the middle region, to develop into the vitellarium, etc.; Fig. 5 is from the outer growing tip, to form the vagina. Hermann, iron hæmatoxylin.

Figs. 6 and 7. From the primary anlage of the same section from which Fig. 1 was taken; Fig. 6 from the middle, Fig. 7 from the outer end. Zenker, Ehrlich-Biondi.

Fig. 8. A case of mitosis in the primary anlage. King, iron hæmatoxylin.

Fig. 9. Mitosis in a pre-oögonium. Zenker, safranin and gentian violet.

Fig. 10. Polar view of a mitosis from the same anlage as Fig. 9.

Fig. 11. Cell from near the growing tip of the ovary anlage; a pre-oögonium. Zenker, Ehrlich-Biondi.

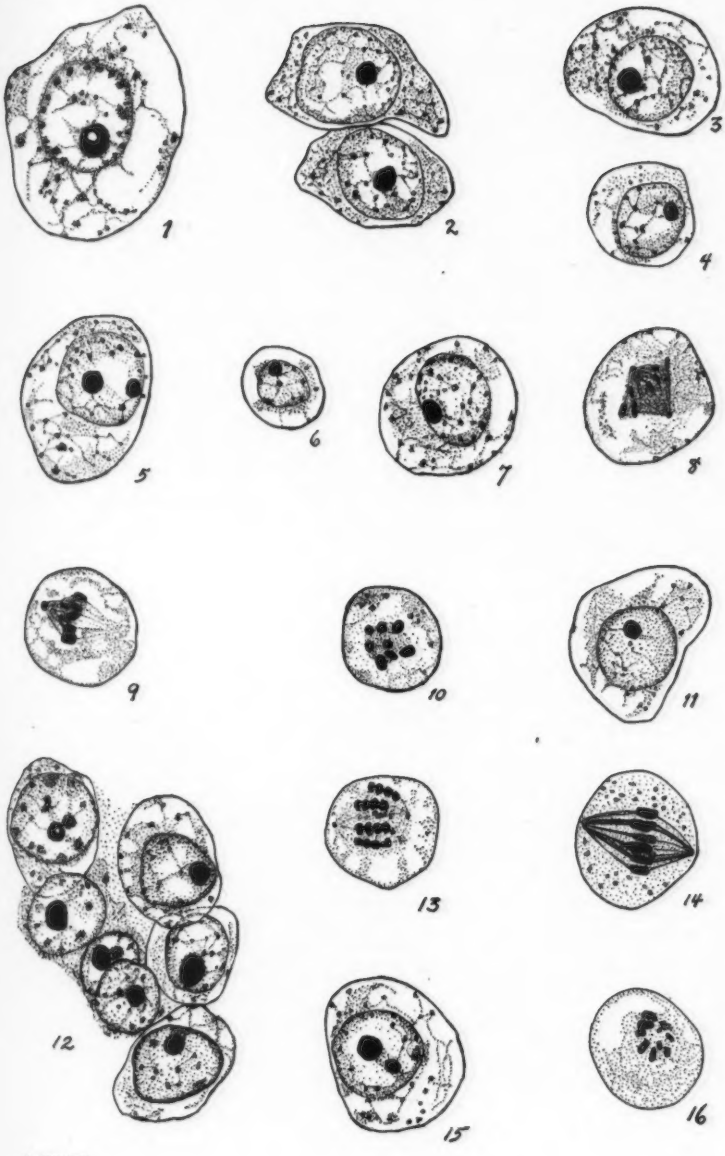
Fig. 12. Group of pre-oögonia just before the formation of the follicular membrane. Cytoplasmic boundaries are not well preserved in all cases. The overlapping of such nuclei as are shown here gives a misleading suggestion of amitosis. King, iron hæmatoxylin.

Fig. 13. A stage in the mitosis of a late pre-oögonium before the condensation of the chromosomes. Hermann, iron hæmatoxylin.

Fig. 14. The most distinct case of mitosis found throughout the oögenesis; spindle fiber bundles are very strong. A late pre-oögonium. King, Ehrlich-Biondi.

Fig. 15. Pre-oögonium, slightly later than Fig. 12. King, iron hæmatoxylin.

Fig. 16. A young oögonium in mitosis; polar view. Zenker, iron hæmatoxylin.



A. RICHARDS

EXPLANATION OF PLATE III.

FIG. 17. Young oögonia, showing three sets of nuclei arranged in pairs. Only one nucleolus is present in each, the large black granular masses being chromatin. Cytoplasmic boundaries not well preserved. King, iron hæmatoxylin.

FIG. 18. Oögonia; the upper nucleus stained very heavily. King, iron hæmatoxylin.

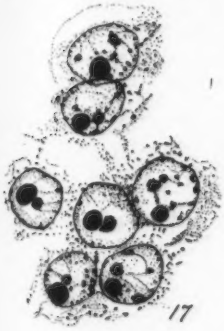
FIG. 19. An oögonium beginning growth. King, iron hæmatoxylin and Bismarck Brown.

FIG. 20. Mitosis in an oögonium just before the growth period begins. King, iron hæmatoxylin.

FIG. 21. An oögonium beginning growth; the chromatin is beginning to condense in preparation for the synapsis stage. King, iron hæmatoxylin.

FIG. 22. Oögonium from the same ovary, slightly later in development.

FIGS. 23–28. Progressive stages of synapsis, 23–26 from the same slide. King, iron hæmatoxylin. 27, King, iron hæmatoxylin. 28, King, Ehrlich-Biondi. In Fig. 26 are to be seen the beginnings of the yolk nuclei. They do not regularly appear at this stage.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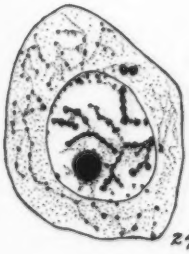
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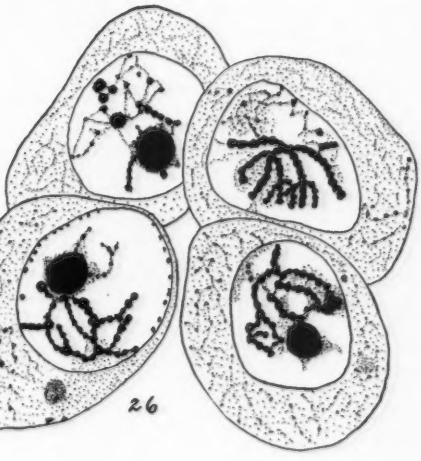
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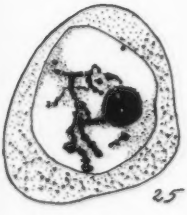
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Explanation of Plate IV.

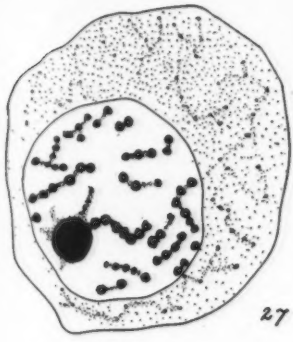
In the latter part of the growth period, following synapsis, yolk is produced (Figs. 31–35) and the nucleoli exhibit certain characteristic features. Figs. 28, 29 and 30, selected from the oöcytes, show a nucleolus proper, paranucleolus, endonucleolus and certain peculiar dark (chromatic?) bodies. These bodies give the characteristic staining reaction of chromatin, very dark with gentian violet. The nucleolus with the triple stain is violet, paranucleolus pink, endonucleolus dark. In the paranucleolus a reticulum supporting the endonucleolus can often be seen (29g).

Fig. 29. King, Ehrlich-Biondi.

Fig. 30. King, triple.

Fig. 31. Oöcyte showing yolk nucleus. The nucleus is not well fixed in this material. Chrom-oxalic, iron hæmatoxylin.

Fig. 32. Sections above the nucleus, passing through the yolk nuclei. Chrom-oxalic, iron hæmatoxylin.



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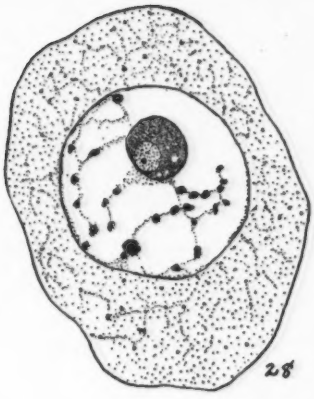

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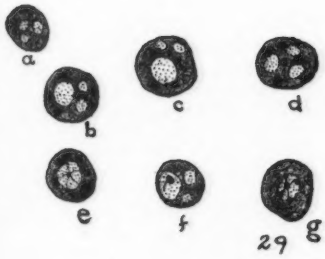

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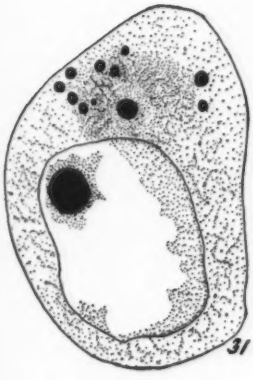

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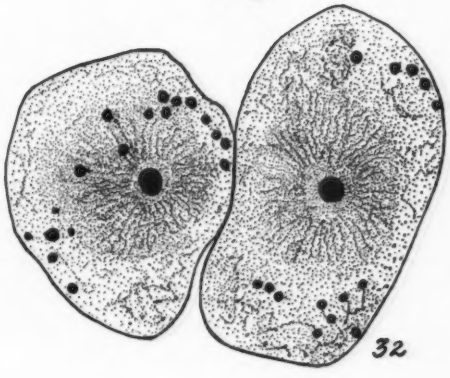

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Explanation of Plate V.

Figs. 33–35. First oöcytes, the ovarian eggs. Yolk globules shown in black in Fig. 35 and indicated by dotted circles in the other figures; chromatin in the characteristic arrangement. Two peculiar black bodies in Figs. 34 and 35 may perhaps be centrosomes or spheres. Fig. 33, King, iron hæmatoxylin; 34, Zenker, Ehrlich-Biondi; 35, King, iron hæmatoxylin.

Figs. 36–50. Development of the vitellarium. Character of the early development is shown in Figs. 4 and 6 from the primary anlage.

Fig. 36. Corresponds to Fig. 11 in time of development. Zenker, Ehrlich-Biondi.

Fig. 37. Same stage as Fig. 12. King, iron hæmatoxylin.

Fig. 38. Growing vitellarium cell from some section as Fig. 15. King, iron hæmatoxylin.

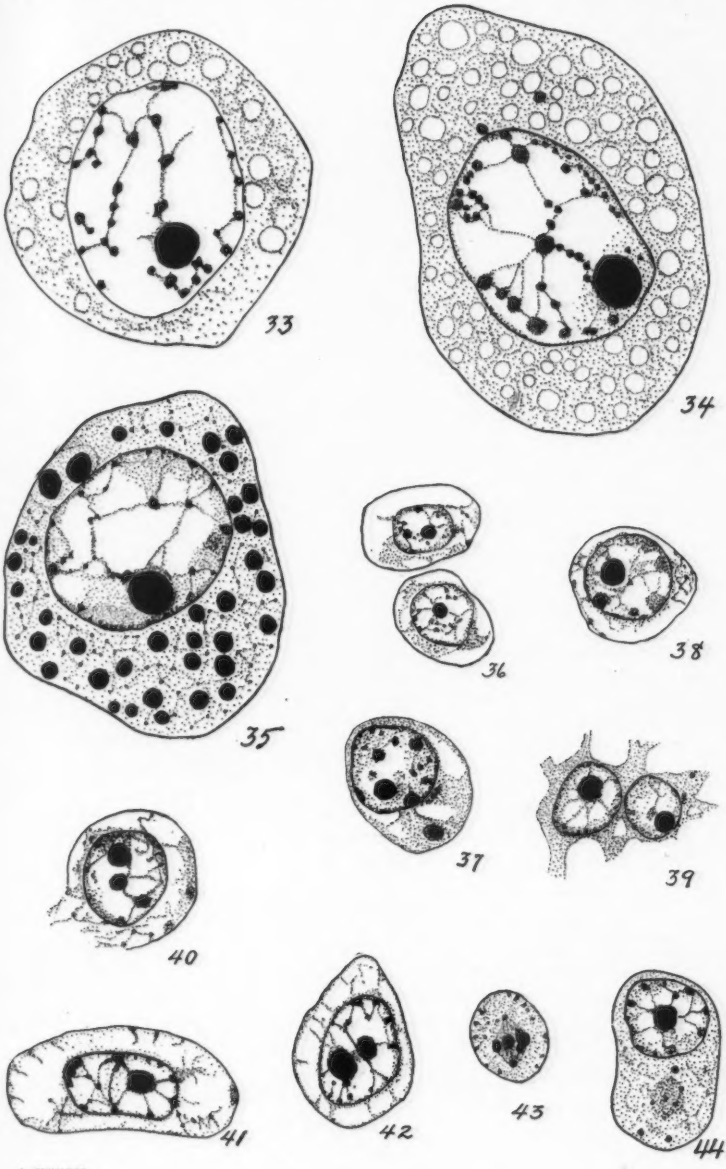
Fig. 39. From the medullary portion of the anlage, the shell gland from the same section as Fig. 37. Cytoplasm drawn out into fibers.

Fig. 40. Shell gland region; same slide as 41 and 42.

Figs. 41 and 42. Vitellarium cells; both cells simulate mitosis due to the heavy linin strands across their centers. Fig. 42 was at first taken for a case of "endogenous division." King, iron hæmatoxylin.

Fig. 43. From growing vitellarium, about the same stage as Fig. 20, a characteristic mitosis. King, iron hæmatoxylin.

Fig. 44. Just before the formation of the yolk body. The dense cytoplasmic mass is probably a yolk nucleus. King, iron hæmatoxylin and Bismarck Brown.



A. RICHARDS

U of M

Explanation of Plate VI.

Figs. 45–49. Stages in the development of the yolk body in the vitellarium cells. King, Conklin's stain.

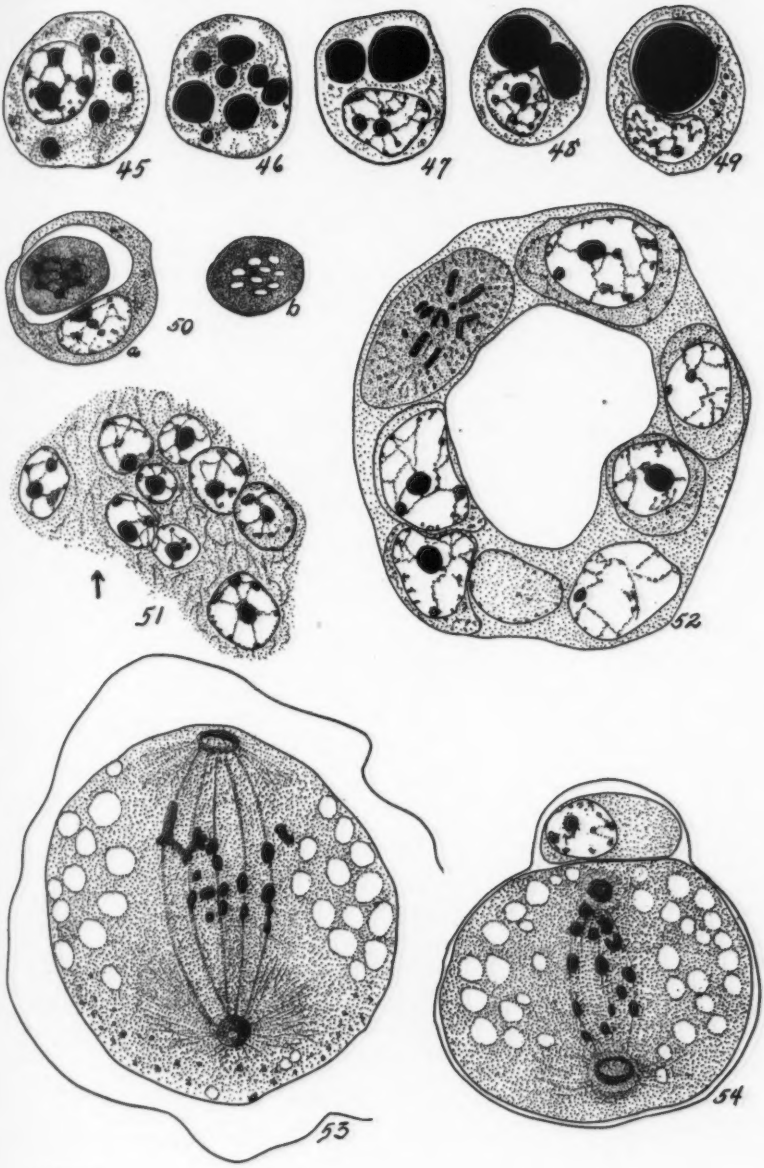
Fig. 50. The appearance of a much decolorized yolk mass. *b* is the same yolk mass shown in *a* at a lower level; probably the central bodies here are remnants of the yolk nucleus. King, iron hæmatoxylin.

Fig. 51. Cells early in the development of the female genital ducts. Cytoplasmic boundaries are not well fixed in this preparation. The nuclei show no evidences of division either direct or indirect. Arrow points in the direction of the axis of the duct. King, iron hæmatoxylin.

Fig. 52. A cross section from a somewhat later stage in oviduct formation. A case of mitosis is shown here. Formol-sublimate, iron hæmatoxylin.

Fig. 53. First maturation division of an oöcyte. King, iron hæmatoxlyin.

Fig. 54. Second maturation division. Zenker, iron haematoxylin.



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Explanation of Plate VII.

Fig. 55. Reconstruction of the female pronucleus; male pronucleus near. The other polar body in the next section. Zenker, Conklin's stain.

In the remaining illustrations the blastomeres not shown in the figure were to be found in the next section.

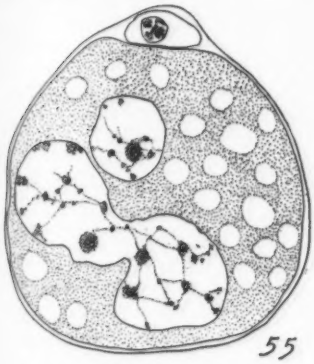
Fig. 56. First cleavage division. Zenker, safranin and gentian violet.

Fig. 57. Division cutting off the second micromere. Three-cell stage. Zenker, safranin and gentian violet.

Fig. 58. Formation of the third micromere. Dotted line indicates the position of the second micromere. Zenker, safranin and gentian violet.

Fig. 59. Four-cell stage, showing reconstruction of macromere nucleus after the last mitosis. Zenker, safranin and gentian violet.

Fig. 60. Formation of the fourth micromere. Zenker, safranin and gentian violet.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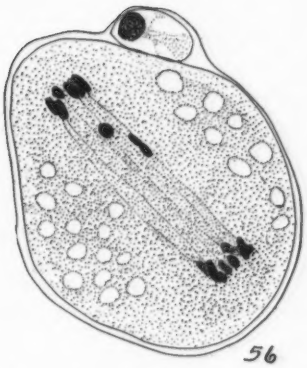
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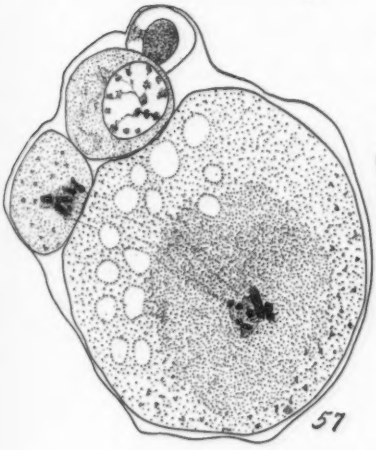
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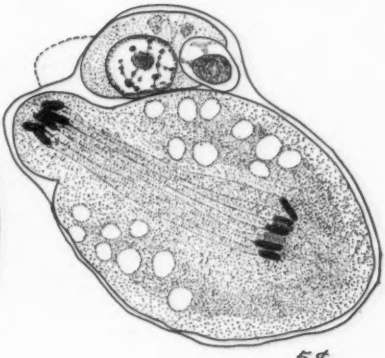
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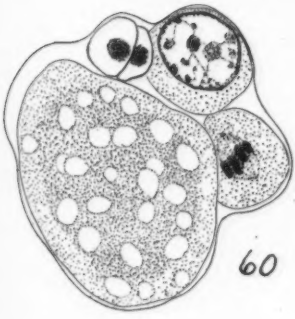
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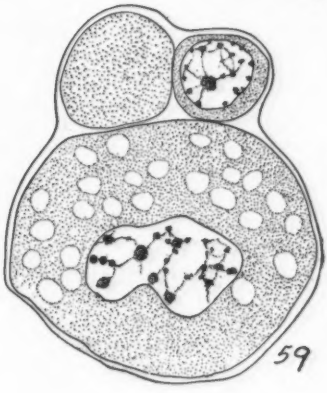
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Explanation of Plate VIII.

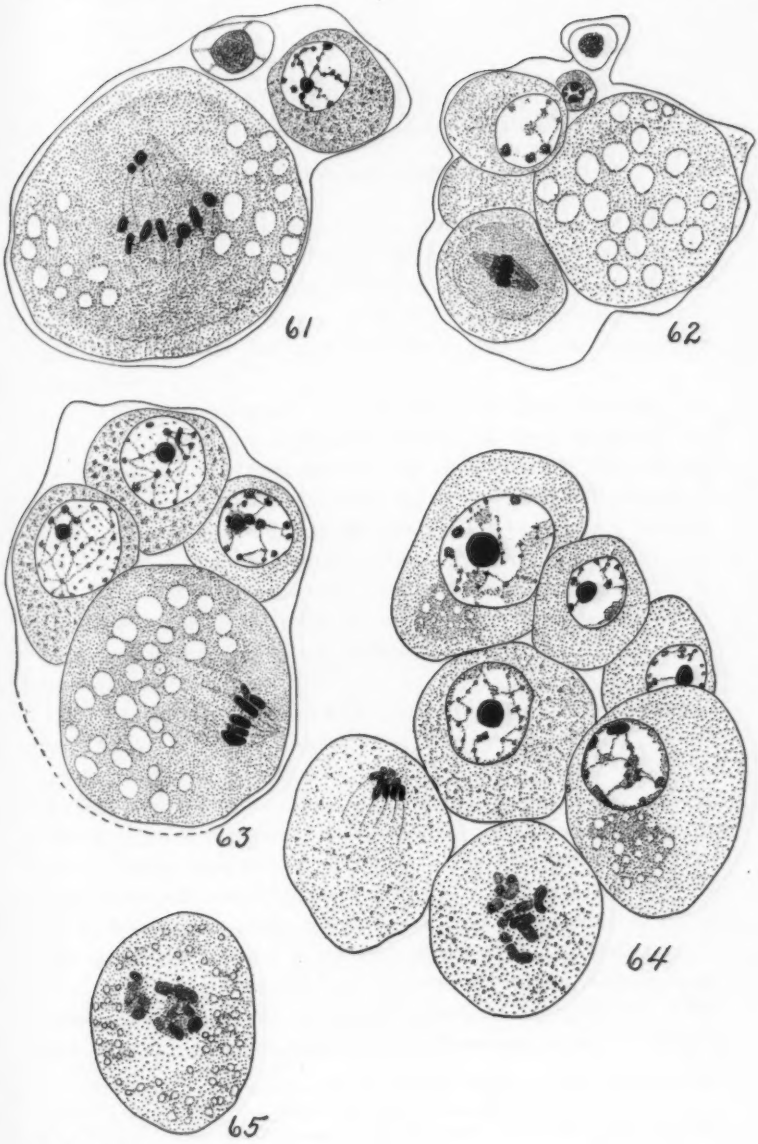
Fig. 61. Six cell stage. Next section shows seven chromosomes belonging to this spindle.

Fig. 62. Seven cell stage. Formol-sublimate, safranin and gentian violet.

Fig. 63. Division in the eight cell stage, only one end of the spindle shown in this section. Zenker, safranin and gentian violet.

Fig. 64. From an embryo of about 30 cells, showing two mitoses. Zenker, safranin and gentian violet.

Fig. 65. A blastomere beginning mitosis from an embryo of 55 to 60 cells. Zenker, safranin and gentian violet.



A. RICHARDS

A NOTE ON THE METAMORPHOSIS OF THE MUSSEL LAMPSILIS LÆVISSIMUS.[1]

ROBERT E. COKER AND THADDEUS SURBER.

While making a collection of the glochidia of various species of mussels used in this laboratory certain observations have been made which prompt the publication of a preliminary note on the peculiar type of glochidium seen in *Lampsilis lævissimus* and other species, and the metamorphosis during the period of parasitism.

1. TYPE OF GLOCHIDIUM AND POSSIBLE SIGNIFICANCE.

The glochidium of *Lampsilis alatus* has been observed by Lefevre and Curtis,[2] who describe it as an "axe-head" glochidium. "This possesses hooks which are not homologous with those of the anodonta type and is to be regarded as more nearly related to the hookless forms, an interpretation which is borne out by the fact that the 'axe-head' can be readily imagined as a modification of the glochidial outline seen in some species of *Lampsilis*, which, like *subrostratus*, show an approach to a rectangular outline."

This glochidium (Figs. 3 and 3*a*) is certainly of a very specialized form. In our laboratory we have observed an almost exactly similar glochidium in *L. capax* (Figs. 4 and 4*a*), while in *L. lævissimus* (Figs. 1 and 1*a*) the same shape is observed but the hooks are wanting. It is remarkable that a larva of so specialized a form should be found in two species such as *alatus* and *capax*, the adult forms of which are so extremely opposed in general shape; *alatus* is one of the most compressed forms in the genus, while *capax* is the most inflated (Figs. 4*b* and 4*c*).

L. lævissimus (Figs. 1*b* and 1*c*) and *L. gracilis* are forms suggestive of *alatus* in the compressed character of the shell. They are somewhat more compressed than *alatus* and are much thinner

[1] Published by permission of the U. S. Commissioner of Fish and Fisheries. Read by title before the American Society of Zoölogists, December, 1910.

[2] "Reproduction and Parasitism in the Unionidæ," *Jour. Exp. Zoöl.*, Vol. IX., No. 1, p. 94.

shelled. These two species seem almost to intergrade (in form of shell) so that one sometimes hesitates in the identification of a specimen of intermediate form. On the other hand, the glochidia of these two species show a striking contrast. The glochidium of *gracilis* (Figs. 2 and 2*a*) is oval-pear-shaped and very similar to that of *ligamentinus* or *ventricosus*, or to what we generally regard as a typical form; while that of *lævissimus* (Figs. 1 and 1*a*) is of the "axe-head" type. Comparison of Figs. 1*a* and 2*a* may suggest, however, that the two species are not so dissimilar as at first appears, and *lævissimus* may be thought of as intermediate between *alatus* and *gracilis*.

The remarkable fact, yet, is that *capax* should have this type of glochidium. *Capax* has always been grouped with *ventricosus* and *ovatus*, but *ventricosus*, at least, has a glochidium of the usual form. Whether the greater significance be attached to resemblances in larval form or in adult form, it is a suggestive revelation of the adaptability of fresh-water mussels and the inadequacy of superficial diagnosis. Undoubtedly in the classification of fresh-water mussels an entirely undue stress has been laid upon characters of a superficial nature. The ultimate system will be based on a much more thorough analysis of the actual anatomy of mussels in young and adult stages than has ever been attempted in systematic work on this group. We have not yet a sufficient knowledge of the comparative anatomy of glochidia to judge of their relative value in the study of relationships, but certain considerations derived from the present case are sufficiently striking to be recorded for their suggestive value.

Ventricosus is certainly closely related to *ligamentinus* and *luteolus*, as judged by the external form. The glochidia, too, are much like those of *ligamentinus*. The adult form is specialized most noticeably in being very inflated. *Gracilis* is specialized in a different way, being exceedingly compressed laterally and having compressed teeth; *lævissimus* (Figs. 1*b* and 1*c*) is a more extreme form of the *gracilis* type, the teeth are more blade-like, while the shell is equally compressed from side to side and has well-developed wings. The glochidium, while suggestive of *gracilis* (which is of the "typical" form), is of the "axe-head" form. The adult *alatus* is of somewhat the same compressed

form, broadly winged, and with "axe-head" glochidia still further modified by the development of hooks. *Capax* has the same type of glochidium, but what of the adult form (Figs. 4*b* and 4*c*)?

In external shape *capax* and *ventricosus* are so closely alike that it has sometimes been doubted if the two species were properly separable. However, the species *capax* is now universally accepted, for the reason that *capax* is clearly distinguishable from *ventricosus* in: (1) the polished almost rayless character of its epidermis, (2) the compressed form of the teeth, and (3) in the relative thinness of its shell, which is inclined to pinkness in color of nacre. *Now these*, it is significant to remark, *are all characters of lævissimus*. In fact, (1) above is the most certain means of distinguishing *lævissimus* from *gracilis*.

If, then, we were to draw an inference from the glochidia as to a relationship between *lævissimus*, *alatus* and *capax*, there would be strong corroborative evidence in the adult characters, in spite of the fact that *lævissimus* and *capax* are the two extremes in degree of inflation. The similar degree of inflation of *capax* and *ventricosus* would offer only a striking instance of convergence in one character.

2. Change of Form during Parasitism.

The change undergone by *Lampsilis lævissimus* during parasitism is most striking. As a general rule, it has been held that there is practically no growth in size during the period of parasitism—simply a metamorphosis from glochidium to young mussel in rudimentary form. Our observations show a notable exception to this general rule.

While examining the gills of a specimen of the sheep's-head, *Aplodinotus grunniens*, several specimens of encysted glochidia were found in an advanced stage of development. The infection occurred in nature, so that the age of the mussels cannot be stated.

Figs. 5, 6 and 7 show not only the striking change in form, but the enormous increase in size as well. Fig. 5 shows a specimen in side view; the "axe-head" glochidium shell is evident, but the mussel is now nearly circular in outline and several times larger than the glochidium. The specimen shows considerable

inflation. Fig. 7 shows the dorsal aspect of a more advanced specimen; the degree of inflation is apparent; the glochidial shell is insignificant in size as compared with the mussel; it is like a narrow saddle which extends only about half way over the side of the mussel shell. The position of the glochidial shell valves in Figs. 5 and 6 will be better understood from a comparison with this figure. The specimen of Fig. 6 is viewed from a ventro-lateral aspect. The following measurements (in decimal parts of millimeter) were made from the specimen represented by Fig. 6:

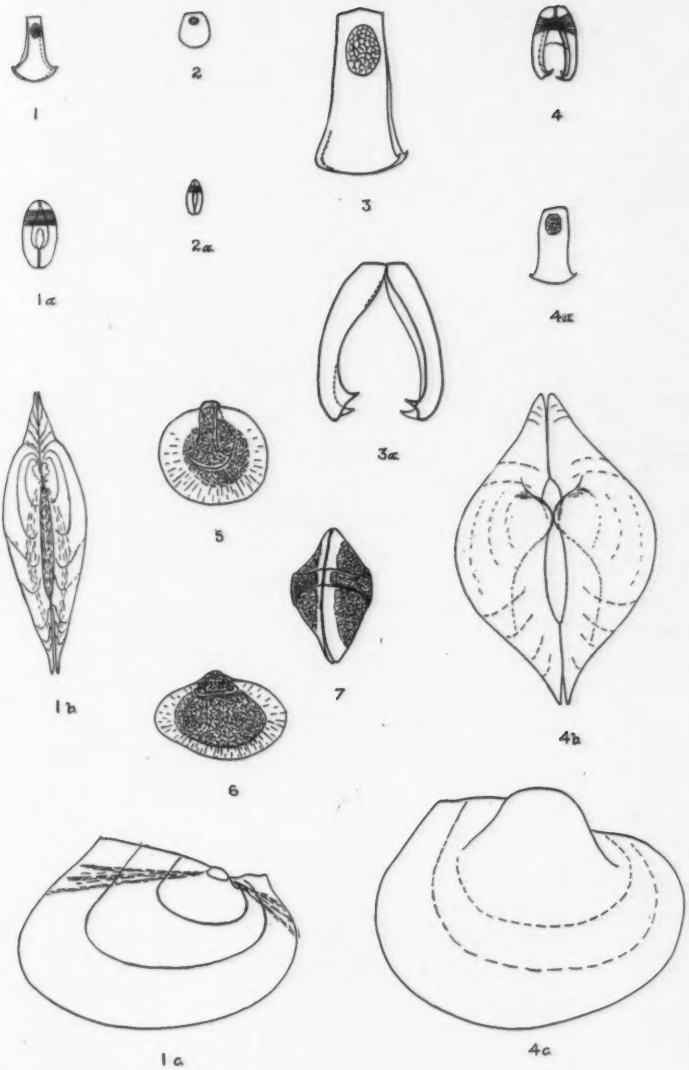
Length of glochidium	0.095 mm.
Width (height)	0.15 mm.
Length of mussel	0.320 mm.
Width (height) of mussel	0.215 mm.

The mussel, as compared with glochidium, is nearly three and one half times as long and nearly one and one half times as wide. It is evident that there must have occurred a material increase in the size of the cellular cyst of the tissue of the host which encloses the young mussel.

The shape of the mussel in the stage shown by Fig. 7 led to the suspicion that the glochidium was of *capax* instead of *lævissimus*, but the form of the glochidium is that of *lævissimus*, and there is no sign of teeth on the shell.

It is not known that such a growth in size and alteration of form occurs in any other species during the period of parasitism. What further change in form or growth in size would occur before the liberation of the mussel cannot be determined without additional material, not now available.

BIOLOGICAL STATION,
FAIRPORT, IOWA,
December 23, 1910.


1
2
3
4
1a
2a
3a
4a
1b
5
7
4b
6
1c
4c

NOTES ON SOME ARACHNIDS FROM OHIO VALLEY CAVES.[1]

NORMAN E. McINDOO.

In September, 1909, immediately after the expiration of my year at the Indiana University's Cave Farm,[2] I spent two weeks collecting arachnids in the following caves: Marengo, Spring, Wyandotte, Little Wyandotte, Sibert's Well, Saltpeter and Mammoth Cave. In all these caves 268 individuals were caught. The expenses in part were defrayed by a grant from the American Association for the Advancement of Science.

Linyphia Weyeri Emerton[3] was taken only from Marengo Cave. This species in this cave is as abundant as *Troglohyphantes* (*Willibaldia*) *cavernicola* Keys. is in the Shawnee Cave. They are most abundant at a place 200 feet from the old entrance, or 500 from the new one. None were seen farther that 1,200 feet from the old entrance. Insects from this locality to the end of the cave are very rare, while near the entrance they are very abundant. The habitat, habits, webs and cocoons of this spider are similar to those of *Troglohyphantes*. Some were collected on their snares in the angles formed by the floor and wall, some along clay banks, some in little pits in the sand floor, and others under rotten boards and débris with thysanurans, beetles, diptera and myriopods. They were not sensitive to my carbide light, but were easily irritated by blowing on the web. The snares were quite abundant and were constructed like those of *Troglohyphantes*. Several cocoons were collected; one contained newly hatched spiderlings which were white in color. One spider was caught eating a thysanuran. Since the entrance to this cave is an artificial one securely made in a sink hole, we may expect an even temperature throughout the cave. And since the spiders are most abundant near the entrance we may attribute this phenomenon to the great number of insects.

[1] Contribution No. 117 from the Zoölogical Laboratory of Indiana University.

[2] "Biology of the Shawnee Cave Spiders," BIOL. BULL., Vol. XIX., No. 6, November, 1910.

[3] These various species were identified by Dr. Alexander Petrunkevitch.

Phanetta subterranea Emerton.—A few were collected 300 feet from the entrance of Spring Cave (a wet cave one fourth mile west of Marengo Cave). These were found under flat rocks and rotten boards on the floor in damp places. They were in company with thysanurans. Several were caught in all parts of Little Wyandotte; however, none nearer the entrance than 75 feet. A few of these were observed under flat stones, but most of them were seen on the base, or in the cracks of stalagmites. Those on the base were usually running about, while those in the cracks were hanging to the underside of their tiny webs.[1] The snare is a flat sheet. It generally droops a little in the center. The meshes are very minute and the number of attachment threads depends on the surroundings. This species is a swift runner and the young are entirely white. Small diptera, thysanurans and myriopods were rather plentiful. This arachnid was abundant in Saltpeter Cave under flat rocks in moist places. They could not be affected by carbide light. Diptera and thysanurans were common.

Anthrobia mammouthia Tellkampf was found only in Mammoth Cave. Eleven specimens were caught. Since I had to keep in company with a crowd of cave visitors who were led by a guide, I was unable to stop more than one or two minutes at a place. However, I found specimens at various localities and undoubtedly they may be taken at any place in the cave where there is sufficient moisture. They were always found under flat rocks, on old mouldy paper and among débris. One small white, disk-like cocoon was observed under a flat stone. These spiders are rather active, not fast runners, and are not difficult to catch for they cling tightly to the rocks and to one's fingers. They vary in color from white to a light brown.[2] In most places insect life seemed to be comparatively scarce. Perhaps these arachnids eat old mouldy paper, remains of lunch and the limited number of small insects.

Meta menardi Latreille.—They are rather common at the mouth of Spring Cave. One was observed in Saltpeter Cave and five were caught at the end of the main channel in Mammoth

[1] Blatchley says these are wandering spiders and spin no webs. "Indiana Caves and their Fauna," Rep. Ind. Geol. Surv., XXI., p. 204.

[2] Hitherto they have always been described as being only white.

Cave. According to the guide this locality is two and one half miles from the entrance. Concluding from the number of webs at this place, *Meta* must be common. Since *Meta* is an outside form and is ordinarily never found very far from the entrance of a cave, there is evidently an opening somewhere near this place. Here as elsewhere in this cave, cave crickets, small diptera and blind beetles were often seen.

Scotolemon flavescens Cope.—This cave harvestman was very abundant in Wyandotte. They were found some distance from the entrance on the damp floor where visitors walk, and often at the base of damp stalagmites. They were always found in the same places as thysanurans and where candle drippings were common. They probably eat the candle drippings and each other, for three couples were seen fighting and several were found dead. They are the least active arachnids I have ever seen and are not affected by carbide light. The second pair of legs, exceedingly long, are used as tactile organs. Several individuals of this species were caught in Little Wyandotte.

Phalangodes armata Tellkampf.—Two specimens of this cave harvestman were taken in Mammoth Cave, one at River Styx and the other at end of main cave. It is much larger and more active than the preceding species. It is usually found on the walls and not on the floor.

Chtonius Packardii Hagen.—One specimen of this small semi-blind pseudoscorpion was taken in Wyandotte, two in Little Wyandotte and one in Mammoth. I have also taken it in Shawnee Cave. It is usually found under damp rocks. It moves along slowly with its chelæ held in the air in front, and is very difficult to find.

In order to keep the specimens from any of these caves alive very long, it was necessary to place them in a saturated atmosphere. They were most conveniently kept in small vials. One individual with a drop of water was placed in each vial. In such confinement several died in a few days, but the majority survived for a month or more. Light experiments like those with *Troglohyphantes* were prosecuted with *Theridium porteri* Banks and *Erigone infernalis* Keys., collected in Mayfield's Cave. They were decidedly negatively phototropic. Various outside forms

were likewise experimented with. Some of these were negatively, others positively phototropic. The kind of phototropism depends on the natural environments of the specimen. It was observed that the specimens collected on this trip always preferred the dark end of the vials. All true cave spiders are more or less negatively phototropic. The distribution and number of all true cave spiders are controlled by even temperature and the abundance of food. They probably have no enemies other than themselves and are rarely seen fighting. They soon die outside the caves unless kept in a saturated atmosphere.

A FURTHER NOTE ON KERATOSUM COMPLEXUM.

CHARLES W. HARGITT.

In my original description of this anomalous hydroid,[1] it was stated that not only was it necessary to create for it a new genus as well as species, but that there "might be the necessity of establishing for it a new family." It was further said: "Concerning the family relations I am not disposed in this connection to enter into any critical review. While the Perisiphonidæ would be the only one under which it might be placed, still this family as at present defined . . . would by no means provide for the species. For example, while there is an axial tubular mass, as shown in Fig. 9, there is no single one of these which bears the hydrotheçæ as called for by the definition referred to. However, the species may be left under this family till such time as adequate revision may be undertaken, when needed modifications may be provided."

I have since found access to a paper by W. Baldwin Spencer, entitled "A New Family of Hydroidea, Together with a Description of the Structure of a New Species of Plumularia," published in *The Transactions of the Royal Society of Victoria*, 1890, a publication of very limited circulation. Had this account been available to me at the time of my perplexity concerning the family relations of *Keratosum* it would have greatly facilitated my insight into many features which were extremely puzzling. I take the first opportunity to add to my earlier account what seems to be a solution of the point left in abeyance pending further knowledge.

Spencer established the family Hydroceratinidæ for a rather remarkable hydroid obtained from Port Phillip having much in common with *Keratosum* in both structure and habit, though with very sharp differences, the details of which need not be given here since only the matter of its family features are in question. One interesting point of coincidence may be cited, namely, that Baldwin at first referred his specimen to the family

[1] BIOL. BULL., Vol. XVII., p. 379.

Ceratelladæ, Gray, thought to belong to the sponges, but later recognized as hydroids. Further comparison convinced the author that his species could not be included under the Ceratelladæ, hence his institution of the Hydroceratinidæ. The following is his definition of the family:

"Family HYDROCERATINIDÆ."

"Hydrophyton consisting of a mass of entwined hydrorhiza, with a skeleton in the form of anastomosing chitinous tubes: the surface is studded with tubular hydrothecæ, into which the hydranths can be completely retracted. Hydranths sessile and connected with more than one hydrorhizal tube, claviform with a single verticil of filiform tentacles. Defensive zoöids present with a solid entodermal axis and nematocysts borne at the distal end."

To one who has any considerable knowledge of hydroid morphology it will hardly be necessary to point out the more obvious features in this definition which directly or approximately embody corresponding features as described in the account of *Keratosum*. There are, however, certain points in which I am not certain that the definition of Hydroceratinidæ would wholly apply to *Keratosum*. For example, it is stated that "hydranths [are] connected with more than one hydrorhizal tube." I have directed attention to the complex anastomoses of the siphonal (this term seems more accurate than Spencer's hydrorhizal) tubes but I have not been able to confirm the condition diagrammatically portrayed by his Figs. 3 and 13. It must be noted, however, that my material was not in that vital condition rendering easy and certain the demonstration of a point like the one in question. Still the tubular and hydrothecal relations were such as to render it perfectly sure that the conditions figured by Spencer are lacking in *Keratosum*.

But with this difference granted it does not seem sufficiently great to vitiate the many points of agreement which are more fundamental and characteristic as family features. There are also apparent differences as to the nematophores of the two species, yet these are rather specific than even generic and may be disregarded in this connection. Furthermore, there is not in

Keratosum any tendency toward a bilaterality in the growth habit such as described for *Clathrozoön wilsoni*. But here again we have a feature which need hardly be considered incompatible in the family aspects of the species. One finds such differences of aspect in genera and species of Gorgonidæ, to which *Clathrozoön* bears some superficial resemblance. I am constrained to believe that in its family relations *Keratosum* is more closely akin to Hydroceratinidæ than to any existing family of *Hydrozoa*, and am quite prepared to propose that it be so designated, at least until stronger reasons are found for a different disposition.

In closing, attention may be called to a still further coincidence between *Keratosum* and *Clathrozoön*, namely, the absence in both of any trace of reproductive organs. I had already emphasized this in the original description of *Keratosum*, stating that while an absence of germ cells might call for no surprise, yet "if gonangia are an organic part of the skeleton one might expect some trace of them, . . . but none could be recognized."

This further inquisition into the morphology of these hydroids tends to confirm and emphasize what had been said in concluding my original description, that "we have in this hydroid one of the most interesting, and in some ways anomalous, of this remarkable group of organisms." The foregoing comparison of the species with that from Australia, *Clathrozoön wilsoni*, tends to further accentuate this impression.

Naples Zoölogical Station,
January 3, 1911.